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Context Dependent Effects of Catecholamines on Human Gut Bacterial Growth and Community Composition

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Abstract

The gut microbiota exists within a complex bidirectional relationship with its host, responding not only to diet but also to host-derived neuroendocrine signals. Catecholamines, including adrenaline, noradrenaline, dopamine, and their precursor levodopa, are stress-associated neurochemicals known to influence bacterial growth and virulence. Yet, understanding direct microbial responses to catecholamines in controlled, multi-species systems remains limited. Key gaps include the concentration-dependence of these effects, the modulatory role of environmental factors such as iron and dietary polymers, and whether single-species observations translate into community-level dynamics.

To address these gaps, this study used an *in vitro* approach using 12 gut-associated bacterial species. A dose-response assay across six catecholamine concentrations revealed highly species-specific, non-linear growth responses across concentrations. Hemin supplementation selectively magnified catecholamine-induced growth in opportunistic taxa with inhibition in several commensals. A polymer-catecholamine assay with six dietary polymers showed that catecholamine effects were substrate-dependent, with the strongest responses observed in FOS (fructooligosaccharide)-containing media. Annotating species' genomes for CAZymes (carbohydrate-active enzymes) showed shared glycoside hydrolase (GH) families in species with significant effects. Finally, 16S rRNA sequencing of the same species in a synthetic community confirmed that catecholamine exposure restructured community composition (PERMANOVA $R^2 = 0.518$, $p = 0.018$), with low-abundance genera such as *Ruminococcus* and *Bifidobacterium* exhibiting the most pronounced treatment-specific shifts. Most importantly, community level effects did not fully mirror the effects seen in monocultures.

Together, these findings demonstrate that catecholamines can act as selective ecological modulators of gut microbial growth, community composition and potential metabolic function in a context-dependent manner.

ZUSAMMENFASSUNG

Die Darmmikrobiota steht in einer komplexen wechselseitigen Beziehung zu ihrem Wirt und reagiert nicht nur auf die Nahrungsaufnahme, sondern auch auf vom Wirt ausgehende neuroendokrine Signale. Katecholamine wie Adrenalin, Noradrenalin, Dopamin und Levodopa sind stressassoziierte Neurochemikalien, die bekanntermaßen das Bakterienwachstum und die Virulenz beeinflussen. Das Verständnis der direkten mikrobiellen Reaktionen auf Katecholamine in kontrollierten, artenreichen Systemen ist jedoch nach wie vor begrenzt. Wichtige Wissenslücken betreffen die Konzentrationsabhängigkeit dieser Effekte, die modulierende Rolle von Umweltfaktoren wie der Verfügbarkeit von Eisen und Nahrungsfasern sowie die Übertragbarkeit von Beobachtungen einzelner Arten auf die Dynamik auf Gemeinschaftsebene.

Um diese Lücken zu schließen, wurde ein In-vitro-Ansatz mit zwölf darmassoziierten Bakterienarten verwendet. Ein Dosis-Wirkungs-Assay zeigte hochgradig artspezifische, nichtlineare Wachstumsreaktionen über sechs Katecholaminkonzentrationen. Eine Häminsupplementierung verstärkte selektiv das durch Katecholamine induzierte Wachstum opportunistischer Taxa, während bei mehreren Kommensalen eine Hemmung auftrat. Ein Polymersubstrat-Assay mit sechs Nahrungspolymeren zeigte substratabhängige Effekte, wobei die stärksten Reaktionen in FOS-haltigen Medien beobachtet wurden. Die CAZyme-Annotation der Genome ergab gemeinsame Glykosidhydrolase-(GH)-Familien bei Arten mit signifikanten Effekten. Schließlich bestätigte die 16S-rRNA-Sequenzierung in einer synthetischen Gemeinschaft, dass die Exposition gegenüber Katecholaminen die Gemeinschaftszusammensetzung veränderte (PERMANOVA R^2 = 0,518; p = 0,018), wobei wenig abundante Gattungen wie Ruminococcus und Bifidobacterium die ausgeprägtesten Verschiebungen aufwiesen. Vor allem spiegelten die Effekte auf Gemeinschaftsebene die in Monokulturen beobachteten Effekte nicht vollständig wider.

Zusammengefasst wirken Katecholamine als selektive ökologische Modulatoren des mikrobiellen Wachstums, der Gemeinschaftszusammensetzung und der potenziellen Stoffwechselfunktion im Darm in kontextabhängiger Weise.

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Chapter 1: Introduction

1.1 The human gut as a dynamic ecosystem

The human gastrointestinal tract is home to a densely populated microbial ecosystem with complex interactions shaped by a constantly changing environment. This ecosystem is composed of tens of trillions of microorganisms including bacteria, archaea, fungi and viruses, collectively called the gut microbiota (Safarchi et al., 2025). The collective genomes of the gut microbiota, referred to as the gut microbiome, exceeds that of its host and plays key roles in health and disease (Afzal et al., 2022). The composition of the gut microbiota is established early in life and undergoes continual changes in response to host development, environmental exposures, dietary habits, medication and lifestyle factors (Thursby and Juge, 2017). This results in a highly dynamic environment that varies among individuals.

Despite this interindividual variability, the gut microbiota exhibits several characteristics commonly associated with complex ecological systems. Similar to other natural ecosystems, the gut microbiota is characterized by diversity, stability, and resilience, which collectively contribute to its ability to maintain functional integrity despite environmental perturbations (Lozupone et al., 2012). Among the many microorganisms inhabiting the gastrointestinal tract, approximately 500–1,000 bacterial species have been identified (Sommer & Bäckhed, 2013). The majority belong to the phyla *Firmicutes* and *Bacteroidetes*, while *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Fusobacteria*, and *Cyanobacteria* are present in lower abundances (Thursby & Juge, 2017; Donaldson et al., 2016).

This remarkable diversity of the gut microbiota is maintained by interactions between these species. Interactions between these species are fundamental to the community structure and function. These interactions may be negative such as competition for limited nutrients or positive such as cooperation whereby one species benefits another (Coyte & Rakoff-Nahoum, 2019; Makki et al., 2018). These complex interactions contribute to the function of the ecosystem within the host despite divergent gut microbiota compositions between individuals (Lozupone et al., 2012).

1.2 The gut microbiota functions as a metabolic interface

One of the key functions of the gut microbiota is the degradation of certain dietary components that are not digested by the host. The discovery of these dietary components were made in 130 A.D. by physician Galen with the observation that certain foods either 'excite' or 'hinder' bowel movements. Specifically, he noted that white bread was 'slower' to pass in the intestine in comparison to brown bread (Jones, 2014). This was the first record that some food components are not fully broken or absorbed by our bodies. Today, these food components namely fibers are defined as a type of carbohydrate that is not digested in the stomach or small intestine (Jones, 2014; Makki et al., 2018). These fibers are derived from multiple sources such as plant cell walls, marine algae, and even microbes. They reach our diet naturally via grains, legume and vegetables or through processed foods. They contain ten or more molecules (monomers) linked together forming a polymer that is resistant to human digestive enzymes [Jones, 2014; Makki et al., 2018; DeVries, 2004]. Furthermore, they belong to the following categories: (1) naturally occurring edible carbohydrate polymers, such as cellulose, hemicellulose and pectins; (2) carbohydrate polymers extracted from raw food materials by physical, enzymatic, and chemical means such as inulin; and (3) synthetic carbohydrate polymers with physiological benefits such as polydextrose (Do Carmo et al., 2016; Jones, 2014; Makki et al., 2018; Fu et al., 2022).

As the definition of dietary fiber expanded to include a broader range of non-digestible carbohydrates, they are further characterized based on their physiochemical properties. These properties also affect the extent to which these complex carbohydrates are accessible to microbial degradation which include their solubility, fermentability, and viscosity, with pectin, inulin, and psyllium as examples of these respective characteristics (Makki et al., 2018). Humans cannot digest most of these dietary fibers because they lack the necessary enzymes to break down these complex carbohydrates (hereafter referred to as polymers) (Makki et al., 2018; Fu et al., 2022). Consequently, many dietary polymers reach the colon largely intact, where they become substrates for microbial fermentation, thereby establishing a symbiotic relationship between the gut microbiota and the host. A

major outcome of this microbial fermentation is the production of short-chain fatty acids (SCFAs) (Makki et al., 2018; Gill et al., 2021; Martens et al., 2014). SCFAs are fatty acids containing fewer than six carbon atoms, with acetate, propionate, and butyrate representing the predominant SCFAs produced in the gut through microbial fermentation of dietary polymers (Makki et al., 2018). These metabolites contribute to host health by serving as an energy source for intestinal cells, maintaining gut barrier integrity, modulating immune function, and reducing inflammation (Makki et al., 2018). The metabolites generated during fermentation vary depending on the dietary polymer consumed and the microbial communities involved in its degradation.

1.3 Polymer breakdown depends on microbial diversity

While the physical and chemical properties of polymers influence accessibility to microbes, not all gut microbes possess enzymatic machinery to break down specific polymers. The ability to degrade dietary polymers is largely determined by carbohydrate-active enzymes (CAZymes), a diverse group of enzymes that enable microbes to break down otherwise indigestible carbohydrates (DeVries, 2004). CAZymes include glycoside hydrolases, glycosyltransferases, polysaccharide lyases, carbohydrate esterases, and carbohydrate-binding modules (Scott et al., 2013; Flint et al 2012; Wardman et al, 2022). Each of these enzymes play a different role in polymer degradation. For example, glycoside hydrolases cleave glycosidic bonds within polysaccharides such as starch, whereas polysaccharide lyases facilitate the breakdown of pectins by cleaving their polysaccharide chains (Flint et al., 2012). Carbohydrate-binding modules do not directly catalyze degradation but instead enhance the efficiency of other CAZymes by targeting them to specific substrates, including cellulose and fructans (Flint et al., 2012; Wardman et al., 2022).

Metagenomic sequencing has revealed that the gut microbiome harbors a higher abundance of CAZymes in comparison to other environments (Kurokawa et al, 2007). However, these enzymes are not distributed uniformly among microbial taxa, resulting in substantial variation in carbohydrate degradation capabilities across species (Fu et al., 2022). For instance, Bacteroides species typically possess an extensive number of CAZymes encoded

in clusters called polysaccharide utilization loci (PULs), allowing them to degrade up to a dozen different polymers (Martens et al., 2014). In contrast, *Bifidobacterium* species possess a more specialized CAZyme repertoires. For instance, *Bifidobacterium* species are particularly adapted to utilize fructans such as inulin and fructooligosaccharides (FOS) through a more specific set of carbohydrate-degrading enzymes (Fu et al., 2022), while *Ruminococcus bromii* is recognized as a keystone degrader of resistant starch (Ze et al., 2012; Makki et al., 2018).

Given the variation in CAZyme repertoires among microbial species, the degradation of dietary polymers often requires the coordinated activity of multiple microorganisms. As most dietary polymers are too large to be transported directly across the bacterial cell membrane, degradation typically begins extracellularly through the action of CAZymes. This process releases smaller oligosaccharides and monosaccharides that can subsequently be imported and metabolized by microbial cells (Rakoff-Nahoum et al., 2014). Importantly, these degradation products are not always consumed by the organism that produced them. Instead, they may become available to neighboring microorganisms, facilitating metabolic interactions known as cross-feeding (Makki et al., 2018; Fu et al., 2022; Wardman et al., 2022).

Cross-feeding allows microbial communities to degrade dietary polymers more efficiently by linking the metabolic activities of distinct species. In these interactions, primary degraders initiate polymer breakdown and release intermediate products that can be utilized by secondary degraders. This cooperative metabolism enhances carbohydrate utilization and promotes the production of beneficial metabolites, including short-chain fatty acids (Makki et al., 2018; Fu et al., 2022; Wardman et al., 2022). As a result, the degradation of dietary polymers depends not only on the enzymatic capabilities of individual microorganisms but also on the collective metabolic capacity and diversity of the gut microbial community.

1.4 Decreased availability of dietary polymers can lead to an imbalance called gut dysbiosis

Given that the degradation of dietary polymers relies on the collective metabolic capabilities of a diverse microbial community, the availability of these substrates plays a critical role in maintaining microbial diversity and function. Any reduction in these polymers can disrupt stability within the gut. Gut dysbiosis refers to the state of microbial imbalance and is characterized by decreased bacterial diversity and impaired microbial function (Makki et al., 2018; Gill et al., 2021; Alou et al., 2016). One of the most well-known factors that contribute to dysbiosis is a low intake of dietary polymers which serve as key fuel sources for gut bacteria (Gill et al., 2021). In the absence of these substrates, bacteria that are substrate-dependent, such as Bifidobacterium and Lactobacillus, decline in abundance (Alou et al., 2016). This loss not only reduces microbial diversity but also impairs the production of short-chain fatty acids (SCFAs) (Shen et al., 2021). As SCFA production declines, it weakens the colonic barrier and increases susceptibility to inflammation and infection (Gill et al., 2021; Alou et al., 2016).

Moreover, as dietary polymers become scarce, some microbes shift to degrading alternative carbon sources such as the glycoprotein-rich mucus layer that acts as a protective and mechanical barrier to pathogens (Gill et al., 2021). These further compromise the gut barrier integrity and increase susceptibility to infections. It may also impair cross-feeding interactions between microbial species. A dysbiotic or imbalanced gut environment is a characteristic of numerous gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and obesity (Gill et al., 2021; Alou et al., 2016). Furthermore, recent studies suggest that gut dysbiosis feeds back into host physiology by altering the production and signaling of hormones and neurochemicals such as serotonin, dopamine, and cortisol (Basnet et al., 2024). Ultimately, the absence of sufficient dietary polymers leads to a chain reaction that affects both the gut microbiota and the host.

1.5 The gut microbiota is not only involved in digestion but also produces and responds to neurohormones and neurochemicals

The gut microbiota and host engage in complex, bidirectional communication through a range of neurochemicals and hormones. Evolutionary studies have revealed that many enzymes involved in human hormone metabolism may have originated from gene transfer in bacteria (Fukui et al., 2018). Furthermore, shifts in the gut microbiota have been associated with changes in neurohormones and neurochemicals levels which affect host behavior, metabolism, and immunity (Basnet et al., 2024). This has been the foundation for the field known as microbial endocrinology that bridges microbiology and endocrinology.

The gut microbiota can directly produce certain neuroendocrine hormones such as noradrenaline, acetylcholine and serotonin that connect the nervous system and endocrine (hormone) system (Fukui et al., 2018). In addition to producing these signaling molecules, the gut microbiota can also stimulate special hormone-producing cells called enteroendocrine cells which signal to the brain through the vagus nerve and influence the hypothalamic–pituitary–adrenal (HPA) axis (Fukui et al., 2018; Tomasello et al., 2018). The HPA axis is the body's stress response system and determines how we react to stress by releasing hormones (Neuman et al., 2015). Two well-established examples of gut-brain communication are serotonin and dopamine. Notably, more than 90% of the body's total serotonin is located in the gastrointestinal tract where it is produced by host enterochromaffin cells (EC). Certain gut bacteria such as *Streptococcus, Escherichia* and *Enterococcus* species can modulate its production by stimulating ECs (Neuman et al., 2015). Similarly, *Bacillus* and *Serratia* are capable of producing dopamine within the intestinal environment. Although this microbial dopamine primarily acts locally in the gut (Albani et al., 2025), it highlights potential connections between gut microbial activity and neurological conditions such as Parkinson's disease, which is characterized by dopamine deficiency (Neuman et al., 2015). Nevertheless, this communication is bi-directional where host-derived neurochemicals and hormones can also influence the gut microbiota. In the 1990s, Mark Lyte and colleagues first confirmed that certain pathogenic bacteria detect and

respond to host-derived catecholamines (stress-related hormones) *in vitro* (Lyte and Ernst, 1992). These catecholamines promoted bacterial growth and virulence in species such as *Escherichia coli, Salmonella enterica*, and *Campylobacter jejuni* (Lyte and Ernst, 1992; Lyte, 2004; Lyte, 2014). These host-microbe interactions illustrate a feedback loop where a disruption in one system can cascade through the other.

1.6 Catecholamines as interkingdom signaling molecules

The ability of catecholamines to influence bacterial physiology depends on the capacity of bacteria to detect and interpret these host-derived signals. Bacteria detect changes in their surroundings through regulatory systems that convert extracellular signals into changes in gene expression. One important example is the two-component system (Nixon et al., 1986), which typically consists of a membrane-bound sensor histidine kinase and a cytoplasmic response regulator. Upon sensing an external stimulus, the histidine kinase auto-phosphorylates and transfers the phosphate group to the response regulator, which subsequently modulates the expression of genes involved in processes such as motility, metabolism, biofilm formation, and virulence (Sperandio et al., 2002; Boukerb et al., 2021). In several Gram-negative enteric pathogens, catecholamines such as noradrenaline and adrenaline function as interkingdom signaling molecules. This phenomenon has been extensively studied through the QseBC two-component system, in which the sensor kinase QseC detects host-derived catecholamines and initiates downstream regulatory responses (Clarke et al., 2006). In enterohaemorrhagic *Escherichia coli* and *Salmonella enterica*, activation of QseC has been associated with increased motility and the expression of virulence-related genes (Sperandio et al., 2002).

Interestingly, QseC is not solely involved in the detection of host-derived catecholamines. It was originally identified as part of a quorum-sensing pathway, a bacterial communication system in which signaling molecules accumulate in response to increasing population density and coordinate collective behaviors such as motility, biofilm formation, and virulence (Miller and Bassler, 2001; Sperandio et al., 2002). Specifically, QseC responds to the bacterial signaling molecule autoinducer-3 (AI-3), allowing bacteria to monitor the

presence of neighboring cells while simultaneously detecting host-derived catecholamines (Clarke et al., 2006). Through this dual-sensing capability, QseC integrates signals originating from both the microbial community and the host, enabling bacteria to adapt their behavior to changing environmental and physiological conditions.

1.7 Catecholamines enhance bacterial growth by increasing iron availability

In addition to functioning as signaling molecules that regulate bacterial gene expression, catecholamines can influence bacterial physiology through non-signaling mechanisms. A key mechanism involves iron acquisition. Iron acts as a critical cofactor for enzymes involved in metabolism, energy production, DNA synthesis, and electron transport (Frawley and Fang, 2014; Schaible and Kaufmann, 2004). In the host, iron tightly sequestered as it is bound to specific proteins like transferrin (TF), lactoferrin (LF) and ferritin (Perraud et al., 2022; Schaible and Kaufmann, 2004). This creates an iron limitation for bacteria. However, bacteria have evolved specialized iron-uptake systems, called siderophores, allowing them to compete for iron within the host (Schaible and Kaufmann, 2004). Siderophores are small, high affinity iron-chelating molecules secreted by bacteria to acquire iron in limiting environments (Perraud et al., 2022). They scavenge ferric iron (Fe^{3+}), which is then recognized by specific bacterial receptors and transported into the cell (Frawley and Fang, 2014; Schaible and Kaufmann, 2004). However, not all bacteria have the ability to produce siderophores. Many gram-negative bacteria like *Escherichia coli* produce siderophores while others lack the biosynthesis pathway entirely (Miethke and Marahiel, 2007).

Although siderophores represent a major bacterial strategy for overcoming iron limitation, bacteria can also exploit host-derived molecules that alter iron availability within the host environment. Among these, catecholamines have been shown to play a vital role in facilitating bacterial access to iron. The catecholamine structure contains a catechol ring that forms complexes with ferric iron allowing them to function as siderophores (Perraud et al., 2022). In the host, catecholamines like noradrenaline have been shown to reduce the iron-binding affinity of host proteins, this liberates them and makes iron more accessible to

bacteria (Freestone et al., 2000). Furthermore, catecholamines can also upregulate the expression of genes involved in siderophore production (Sandrini et al., 2010). This dual role of catecholamines to both liberate and enhance iron acquisition provides an advantage for gut bacteria especially when catecholamine levels are elevated during host stress conditions.

1.8 Beyond individual bacterial responses, catecholamines influence microbial community composition

While the composition of a healthy gut microbiota varies considerably among individuals, there are some characteristics that are considered generally favorable (healthy) or detrimental (dysbiosis). An individual's gut microbiota is considered healthy when it supports metabolic functions, resistance to pathogen colonization and production of beneficial metabolites. Where dysbiosis could lead to impaired metabolic function, loss of beneficial species and increased susceptibility to inflammation and disease (Karl et al., 2018). As catecholamines present a competitive advantage to certain bacterial species, they can promote differential growth within microbial communities, leading to shifts in community composition.

Several opportunistic and pathogenic bacteria, including *Escherichia coli*, exhibit enhanced growth, motility, and virulence in response to catecholamines (Freestone et al., 2000; Lyte, 2014). Consequently, these organisms may proliferate more rapidly under conditions associated with elevated catecholamine concentrations. In studies quantifying intestinal microbiota of young monkeys exposed to stressors, a significant reduction of Lactobacilli and Bifidobacteria were seen (Bailey et al., 2010). Coupling these findings highlights how stress-associated catecholamine can shift microbial abundance towards more pathogenic or stress-adapted populations.

These changes in microbial populations have consequences for interactions between species in a community. The gut microbiota is a complex network of interactions which include both cooperation and competition such as cross-feeding. However, with the shift

towards abundant opportunistic bacteria and reduction of beneficial fermenters can interrupt metabolic chains and decrease SCFA production. In line with Coyte et al. (2015), competitive interactions may contribute to a more stable community but a disruption in cooperative interactions that support metabolic efficiency. These changes in community composition have important ecological consequences, including reduced microbial diversity, disruption of cooperative metabolic interactions, and impaired short-chain fatty acid production that could lead to clinical implications.

1.9 Gut microbial community shifts link stress responses to increased disease susceptibility

Among the factors capable of driving microbial dysbiosis, host stress responses have received considerable attention. During prolonged periods of physiological or psychological stress, elevated levels of catecholamines can not only act on host tissues but also on the composition and behavior of microbes favoring pathogenicity and disrupting homeostasis (Peter et al., 2018). As discussed in previous sections, catecholamines can alter bacterial physiology directly through regulatory signaling pathways and indirectly by increasing iron availability. These effects may selectively promote the growth of responsive bacterial taxa while disadvantaging others, leading to shifts in microbial community structure.

Such changes can have important consequences for host health. A healthy gut microbiota contributes to colonization resistance, a process whereby resident microorganisms limit the establishment and proliferation of pathogens (Buffie and Pamer, 2013). However, stress-associated reductions in beneficial commensal bacteria, such as *Lactobacillus* species, may weaken this protective function while allowing opportunistic microorganisms to expand (Bailey et al., 2010). Consequently, individuals experiencing chronic stress may become more susceptible to gastrointestinal infections and pathogen colonization.

Beyond colonization resistance, stress-induced alterations in microbial community composition can disrupt important metabolic functions. Reductions in fiber-degrading and short-chain fatty acid-producing microorganisms may impair SCFA production, thereby

compromising gut barrier integrity and increasing inflammatory responses (Gill et al., 2021; Alou et al., 2016). Such disturbances have been associated with chronic gastrointestinal disorders, including irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), both of which link stress and microbial dysbiosis (Gill et al., 2021).

Moreover, since the gut-brain axis is a bidirectional talk, disruptions in the gut community can cascade beyond the gastrointestinal tract. A broader systemic impact includes altered immune regulation, metabolic processes, and neuroendocrine signaling (Neuman et al., 2015; Fukui et al., 2018). Taken together, these findings reinforce the clinical relevance of catecholamine-microbiota interactions.

1.10 Research gaps and study rationale

Although there is growing evidence of the effects of catecholamines on gut bacterial growth, behavior, and composition, how these effects occur remain unclear. Most existing studies have focused on *in vivo* approaches, but it is difficult to distinguish direct microbial responses from indirect effects. As a result, it is unclear how individual gut bacterial species respond to catecholamines under controlled conditions. In addition, the relevance of catecholamine concentrations are not well-established, particularly at low concentrations.

Furthermore, the interactions between the environment and catecholamines are unexplored. Factors such as iron and dietary polymers are likely to influence bacterial responses. However, these effects have not been systematically investigated. Given that catecholamines can influence iron acquisition and metabolism pathways, understanding how these variables can modulate bacterial growth is fundamental.

Finally, there is limited knowledge between single-species findings translating to communities. Observations made on a single species may be restructured by the complex interactions like competition and cooperation that are found in a community. Therefore, catecholamine effects on a single species level may significantly alter on a community level. Bridging the gap between monocultures and community level dynamics is therefore critical in understanding the ecological impact of catecholamines in the gut.

To address these gaps, this study aims to investigate how catecholamines influence gut bacterial growth across varying environmental contexts including iron and dietary polymers. Moreover, responses in individual bacterial species will also be compared to dynamics in a synthetic community to better understand how catecholamines shape bacterial growth and community composition.

Chapter 2: Materials

2.1 Bacterial Species

The bacterial species used were selected to represent key members of the human gut microbiota which included commensal gut bacteria and species with known opportunistic pathogenic potential. A total of 12 bacterial species were included as listed below in Table 1:

Species	**Strain ID or Source**	**Ecological / clinical relevance**
Bacteroides thetaiotaomicron	DSM2079	Commensal gut bacterium
Bacteroides ovatus	DSM1896	Commensal gut bacterium
Bacteroides caccae	DSM19024	Commensal gut bacterium
Bifidobacterium stercoris	DSM19555	Commensal gut bacterium
Escherichia coli	DSM18039	Facultative anaerobe; opportunistic pathogen
Enterococcus faecium	Gut isolate	Opportunistic pathogen
Enterococcus mundtii	DSMZ 4838	Commensal / opportunistic
Eubacterium ventriosum	Human gut isolate	Commensal gut bacterium
Ruminococcus gnavus	DSMZ 108212	Commensal gut bacterium
Ruminococcus lactaris	Human gut isolate	Commensal gut bacterium
Bacteroides finegoldii	DSM17565	Commensal gut bacterium
Klebsiella pneumoniae	Clinical isolate	Opportunistic pathogen

Table 1: Bacterial species used in this study. Classifications reflect general ecological or clinical relevance and do not imply pathogenic behavior under the experimental conditions used in this study.

2.2 Media and Solutions

All general media and solutions used were prepared according to Table 2 to Table 7 and were autoclaved and stored at room temperature until further use.

2.2.1 Brain Heart Infusion Broth (BHI)

Hemin (0.5 mg/ml), Vitamin K1 (1:100), L-Cysteine, and $NaHCO_3$ were added after autoclaving.

Chemical	Company & catalog number (CN)	Amount
BHI broth	Oxoid; CN: CM1135	37 g
Yeast extract	Oxoid; CN: LP0021	5 g
Hemin (0.5 mg/ml)	Sigma-Aldrich; CN: 51280	10 ml
Vitamin K1 (1:100)	Carl Roth; CN: 3804	5.2 ml
L-Cysteine (stock solution)	Carl Roth; CN: 3468	10 ml
$NaHCO_3$	Sigma-Aldrich; CN: S6297	10 ml

Table 2: Brain Heart Infusion (BHI) medium (undiluted 1L)

2.2.2 L-cysteine stock solution

The L-cysteine stock solution (10% w/v) was prepared according to Table 3 and filtered using a 0.22 µm Sartorius Minisart® non-pyrogenic filter. It was aliquoted and stored at -20°C until further use.

Chemical	Company & catalog number (CN)	Amount
L-Cysteine	Carl Roth; CN: 3468	0.50 G
MilliQ® Water	-	5 ml

Table 3: L-cysteine stock solution (5 mL)

2.2.3 10x Phosphate Buffered Saline (PBS)

Chemical	Company & catalog number (CN)	Amount
NaCl	Carl Roth; CN: 3957	80 g
KCl	Carl Roth; CN: P017	2 g
Na_2HPO_4*H2O	Carl Roth; CN: 4984	17.80 g
KH_2PO_4	Sigma-Aldrich; CN: P5655	2.40 g

Table 4: 10x Phosphate Buffered Saline (PBS, 1L) at a pH of 7.4

Chemical	Company & catalog number (CN)	Amount
10x PBS	-	100 ml
MilliQ® Water	-	900 ml

Table 5: 1x Phosphate Buffered Saline (PBS, 1L)

2.2.4 Gifu Anaerobic Broth (GAM)

Chemical	Company & catalog number (CN)	Amount
Gifu Anaerobic Broth	Himedia; CN: M1801	59 g
MilliQ® Water	-	1000 ml

Table 6: Gifu Anaerobic Broth (GAM, 1L) undiluted

2.2.5 Diluted VL6 Medium

For experimental conditions under hemin-free, VL6 medium without glucose was prepared as Table 7, however, hemin was deliberately omitted from the formulation.

Chemical	Company & catalog number (CN)	Amount
VL6 Medium	Formedium; CN: FOR-VL60201	200 ml
1x PBS	-	800 ml

Glucose	Carl Roth; CN: X997	10 g
Hemin	Sigma-Aldrich; CN: 51280	5 mg

Table 7: Diluted VL6 Medium (1L)

2.2.6 Dietary polymers

Each of the polymers were prepared individually in GAM media at a concentration of 1 g/L (see Table 8) to final volume of 1L.

Chemical	**Company & catalog number (CN)**	**Amount**
Amylopectin	Sigma-Aldrich; CN: 10120	1 g
FOS	Sigma-Aldrich; CN: F8052	1 g
Inulin	Alfa Aesar; CN: A18425	1 g
Pectin	Carl Roth; CN: 8911	1 g
Pullulan	Alfa Aesar; CN: J66961	1 g
Starch	Sigma-Aldrich; CN: S9765	1 g

Table 8: Dietary Polymers (1L)

2.2.7 Catecholamine media for dose response assay

The catecholamines listed in Table 9 and 10 were freshly prepared at a concentration of 1mg/ml for each experiment and protected from light to minimize oxidative degradation. Once dissolved, the solutions were filter sterilized using 0.22 µm Sartorius Minisart® non-pyrogenic filters and transferred into the anaerobic chamber approximately 24 hours prior to inoculation. The relevant hormone concentrations for the dose-response assay were generated by serial dilution (see Table 8) and are reported in mass-based units, with corresponding molar concentrations derived from molecular weight. All catecholamine preparations and serial dilutions were performed identically for both VL6 medium supplemented with hemin and VL6 medium without hemin.

Chemical	Company & catalog number (CN)	Molecular weight	Molar concentration at 1 mg/mL
L-3,4-dihydroxyphenylalanine (DOPA / Levodopa)	Merck; CN: PHR1271	197.2 g/mol	≈ 5.1 mM
Dopamine hydrochloride	Merck; CN: H8502	189.6 g/mol	≈ 5.3 mM
Norepinephrine	Merck; CN: A7257	169.2 g/mol	≈ 5.9 mM
Epinephrine	Merck; CN: E4250	183.2 g/mol	≈ 5.4 mM

Table 9: Catecholamines stock solutions for dose response assay were prepared at 1 mg/ml by dissolving in the relevant diluted VL6 medium (with or without hemin)

Final Concentration (mg/mL)	Dilution factor	Volume from previous dilution	Volume of VL6 medium
0.1	1:10	333 µl	3 ml
0.01	1:10	333 µl	3 ml
0.001	1:10	333 µl	3 ml
0.0001	1:10	333 µl	3 ml
0.00001	1:10	333 µl	3 ml
0.000001	1:10	333 µl	3 ml

Table 10: Serial 1:10 dilutions were used to prepare catecholamine working concentrations from a 1 mg/mL stock solution for the dose response assay. Each dilution was prepared by transferring 333 µL of the previous concentration into 3 mL of VL6 medium. This was done for both VL6 mediums (with and without hemin).

2.2.8 Catecholamine-Polymer Media

Chemical	Stock concentration	Volume added	Final concentration
Catecholamine	1 mg/mL (in PBS)	20 µL	1 µg/mL (0.0001% w/v)
Polymer-containing GAM medium	—	19.98 mL	1 mg/mL = 0.1% (w/v)

Table 11: Hormone-polymer media used in hormone-polymer assay

Chapter 3: Methods

3.1 Hormone Dose-Response Growth Assay

3.1.1 Bacterial species and overnight culture preparation

Bacterial species (listed in table 1) were inoculated from cryopreserved stocks stored at -80 °C. These were initially cultured in Brain Heart Infusion (BHI: Oxoid) media. This rich media was used exclusively for overnight culture (ONC) to facilitate biomass accumulation prior to dose-response assay.

Cultures were prepared in polystyrene culture tubes and grown overnight at 37 °C under strictly anaerobic conditions (85% N_2, 10% CO_2, 5% H_2) in an anaerobic chamber (Coy Laboratory Products, USA). Following overnight growth, 1ml of each culture was centrifuged in 1.5 ml Eppendorf tubes at 11,300 x g for 3 minutes. The supernatant was discarded and the cell pellet was resuspended in 1ml sterile phosphate buffered saline (PBS). The optical density at 600 nm (OD_{600}) was measured, and the cell suspensions were adjusted to a starting OD_{600} of 0.002 prior to inoculation into growth assays.

3.1.2 Preparation of 96 well plates

Growth assays were conducted in flat-bottom 96 well plates (Greiner Bio-One, Germany). For each condition, 190 µL of diluted VL6 growth media (with or without hemin) containing the appropriate catecholamine concentration was dispensed into each well. This step was done under the laminar hood, and the plates were left for approximately 24 hours in the anaerobic tent prior to inoculation with the bacterial cultures. The following day, 10 µL of the normalized bacterial suspension (OD_{600} = 0.002) was added.

All species and conditions were assessed in three biological replicates, each of which was done on a different day. Plates were prepared inside the anaerobic chamber and sealed with Breath-Easy gas-permeable membranes (Merck, Germany, Cat. No. Z380059).

3.1.3 Anaerobic incubation and optical density measurements

Plates were incubated anaerobically at 37 °C in an incubator with an automated plate stacker (EMBL, Germany). Optical density measurements were acquired using an Epoch 2 microplate reader (BioTek) within the anaerobic incubator. Automated readings were conducted with 10s agitation prior to each measurement and OD_{600} was measured at 20-minute intervals over a total duration of 48 h.

3.1.4 Data processing, normalization, and visualization

The raw optical density measurements were exported from the plate reader in Microsoft Excel format and then analyzed using a Python 3-based software package called AMiGA (Analysis of Microbial Growth Assays). This software utilizes Gaussian process regression to model microbial growth curves. AMiGA, then, uses these fitted growth curves to calculate multiple growth parameters in an Excel file. This file served as the input file for downstream analyses in R (version 4.4.2), which included data processing, normalization, statistical comparisons, and visualization. Only the linear area under the curve (auc_lin) was used for further analysis

Data processing and visualization were performed using the R packages dplyr, tidyr, janitor, forcats, ggplot2, scales, and purrr. For normalization of the data, catecholamine-treated data were expressed relative to the media-only control by subtraction. Specifically, for each species and metric used, normalized values were then calculated as:

$$\Delta = X_{\text{Treatment}} - X_{\text{Control}}$$

where $X_{\text{Treatment}}$ is the observed metric value under catecholamine exposure and X_{Control} is the corresponding media-only baseline. This normalization step was conducted for both −Hemin and +Hemin conditions. Heatmap color scales represented control-subtracted values (Δ) using a diverging color palette centered at zero.

In addition to species-level heatmaps, genus-level enrichment analyses were performed by grouping species according to genus and comparing catecholamine-treated samples with matched controls. Enrichment factors (EFs) were calculated as:

$$EF = \left(\frac{2 \times Mean_{treatment}}{Mean_{treatment} + Mean_{control}}\right) - 1$$

where positive values indicate enrichment relative to control and negative values indicate depletion. Dilutions collapsed prior to genus-level enrichment analysis.

3.1.5 Statistical analysis

Statistical comparisons were conducted on the raw (unnormalized) data. For each species, metric, hormone, and concentration, an unpaired two-sample Welch t-test was performed comparing raw hormone-treated values to the raw control values for the same species and metric. Statistical significance was encoded using asterisks based on the resulting p-values ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$= ***). No normalization or transformation was applied prior to statistical testing.

For genus-level enrichment analyses, data from all tested concentrations were pooled (Supplementary Figures S1 and S2). Differences between treatment and control groups were assessed using Wilcoxon rank-sum tests. P-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) correction, and genera with an adjusted p-value < 0.05 and a positive enrichment factor were considered significantly enriched (Supplementary Table S1).

3.2 Hormone-Polymer Growth Assay

3.2.1 Bacterial species

11 bacterial species were included in the hormone-polymer growth assay (Table 1; excluding *Enterococcus faecium*). Prior to the main experiment, a preliminary screening was performed to confirm the ability of each species to grow in polymer containing media. This screening consisted of a basic optical density (OD_{600}) measurement to assess detectable

growth in the presence of polymers. One species, *Enterococcus faecium,* included in previous and subsequent assays was excluded from the polymer assay due to the absence of measurable growth during this screening step.

3.2.2 Overnight culture preparation

For the assay, cultures were prepared in polystyrene culture tubes containing Gifu Anaerobic Broth (GAM) media and grown overnight at 37 °C under strictly anaerobic conditions (85% N_2, 10% CO_2, 5% H_2) in an anaerobic chamber (Coy Laboratory Products, USA). Cells from overnight cultures were diluted in 1:1000 dilution of GAM media containing the relevant polymers and grown overnight again in the same anaerobic conditions. To standardize the inoculum and reduce background nutrients, 10 µL of the overnight culture was further diluted into 990 µL of sterile PBS. This dilution step minimizes residual nutrient carryover from the rich GAM media, ensuring that any observed bacterial growth is more directly attributable to the polymer and/or hormone present.

3.2.3 Growth assay setup

Growth assays were conducted in flat-bottom 96 well plates (Greiner Bio-One, Germany). For each condition, GAM media with the appropriate polymer and hormone was dispensed at a volume of 190 µL. This preparation was done under a laminar hood and left overnight in the anaerobic tent for approximately 24 hours prior to the inoculation with the overnight bacterial cultures.

The next day, 10 µL of the bacterial overnight cultures were dispensed into each well and sealed with Breath-Easy gas-permeable membranes (Merck, Germany, Cat. No. Z380059).

3.2.4 Anaerobic incubation and optical density measurements

Plates were incubated anaerobically and OD measurements were taken for 48 hours as per section 3.1.3.

3.2.5 Growth curve analysis

The raw optical density measurements were exported from the plate reader in Microsoft Excel format and then analyzed using a Python 3-based software package called AMiGA (Analysis of Microbial Growth Assays) (Midani et al., 2021). This software utilizes Gaussian process regression to model microbial growth curves. AMiGA, then, uses these fitted growth curves to calculate multiple growth parameters in an Excel file. This file served as the input file for downstream analyses in R (version 4.4.2), which included data processing, normalization, statistical comparisons, and visualization. Only the linear area under the curve (auc_lin) was used for further analysis

3.2.6 Normalization for visualization

To facilitate visual comparison across polymers, species, and hormones, data were normalized. For each species, as before, the following normalization was performed:

$$\Delta = X_{\text{Treatment}} - X_{\text{Control}}$$

where $X_{\text{Treatment}}$ is the observed metric value under catecholamine and polymer exposure and X_{Control} is the corresponding polymer-only baseline. This normalization step was used to facilitate visualization only and not used for statistical testing. All statistical testing was conducted on unnormalized replicate means.

To investigate broader taxonomic responses, species-level data were aggregated to the genus level. Genus-level enrichment analyses were performed, and enrichment factors (EFs) were calculated as:

$$EF = \left(\frac{2 \times Mean_{treatment}}{Mean_{treatment} + Mean_{control}}\right) - 1$$

where positive values indicate enrichment relative to control and negative values indicate depletion. Bubble plots were generated to visualize enrichment patterns, with bubble size representing the mean control growth value and color indicating the enrichment factor.

3.2.7 Identification of carbohydrate-active enzymes (CAZymes) based on genomic data.

To understand how bacteria grow under polymer-supported conditions, we looked at the genomic potential of these bacteria to process carbohydrates. We did this by identifying carbohydrate-active enzymes (CAZymes) in 11 bacterial species included in this assay using a standard annotation workflow based on the dbCAN pipeline (Yin et al., 2012).

Complete or high-quality genome assemblies were downloaded for each bacterial species from the NCBI genome database in FASTA format. For each genome, open reading frames were distinguished and translated into amino acid sequences. These were generated into protein FASTA (.faa) files and used for downstream CAZyme annotation.

For CAZyme annotation, the dbCAN framework was used. The dbCAN framework annotates carbohydrate active enzymes based on the hidden Markov model (HMM) profiles. The protein sequences generated earlier were scanned against the dbCAN HMM database (dbCAN-HMMdb-V14) using HMMER (v3.3). The detected CAZymes were classified into their respective functional classes according to the CAZy database, which included glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases (PL), carbohydrate esterases (CE), carbohydrate-binding modules (CBM), and auxiliary activity enzymes (AA). The CAZyme annotations were summarized by counting the total number of predicted genes for each CAZyme class and only the five primary CAZyme family classes (GH, GT, PL, CE, CBM) were used for visualization.

3.2.7 Statistical analysis

Statistical comparisons were conducted on the raw (unnormalized) data. For each species, metric, hormone, and concentration, an unpaired two-sample Welch t-test was performed comparing raw hormone-treated values to the raw control values for the same species and metric. Statistical significance was encoded using asterisks based on the resulting p-values ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$= ***). No normalization or transformation was applied prior to statistical testing.

For genus-level enrichment analyses, enrichment factors were calculated from mean treatment and control values. Statistical significance of enrichment was evaluated using Welch's t-tests and resulting p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) correction.

3.3 Synthetic Community Assay

In contrast to the monoculture assays above, this experiment evaluated hormone effects at the community level using 16S rRNA sequencing as a readout of relative species abundance in a mixed community.

3.3.1 Bacterial species and overnight culture preparation

12 bacterial species (see Table 1) were inoculated in VL6 media with hemin in polystyrene culture tubes and grown overnight at 37 °C under strictly anaerobic conditions (85% N_2, 10% CO_2, 5% H_2) in an anaerobic chamber (Coy Laboratory Products, USA) for overnight cultures. Following overnight growth, the cells were washed and normalized as per section 3.1.1. 50 µL of each species was mixed in a 5 ml falcon tube and vortexed shortly.

3.3.2 Preparation of 96 well plates

Growth assays were conducted in flat-bottom 96 well plates (Greiner Bio-One, Germany). For each condition, 190 µL of diluted VL6 growth media with hemin containing catecholamine concentrations of 100 ng/ml was dispensed into each well. This step was done under the laminar hood, and the plates were left for approximately 24 hours in the anaerobic tent prior to inoculation with the bacterial cultures. The following day, 10 µL of the normalized community was added. Each condition contained 2 wells per condition resulting in 400 µL of sample volume per condition.

Each species and condition was assessed in four biological replicates. The plates were prepared inside the anaerobic chamber and sealed with Breath-Easy gas-permeable membranes (Merck, Germany, Cat. No. Z380059).

3.3.3 Community growth and sampling

Plates were incubated anaerobically and OD measurements were taken for 48 hours as per section 3.1.3. After 48 hours, the plate was taken out, and the 2 wells of duplicate samples were mixed in a 1.5 ml Eppendorf tube. To allow community stabilization, 2 µL of these cultures were serially passaged into a 96 well-plate with fresh media for a total of five transfers over the period of 5 consecutive days. At each passaging/transfer, the remaining culture was centrifuged at 11,300 x g for 3 minutes, the supernatant was discarded and the pellet was frozen at -20 °C until 16S rRNA gene amplification and sequencing.

3.3.4 16S rRNA gene amplification and sequencing

The microbial community pellets harvested at the last (5^{th}) timepoint were sent to the Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna, for DNA extraction and 16S rRNA amplicon sequencing (project ID JMF-2602-01). Amplicon sequencing targeted the V3-V4 region of the 16S rRNA gene.

3.3.5 Data processing and analysis

All data processing, statistical analyses and visualizations were performed using R packages dplyr, tidyr, tibble, stringr, phyloseq, Biostrings, vegan, and ggplot2.

Processing of the raw reads involved quality filtering, denoising, paired end read merging, and chimera removal. Following this, amplicon sequence variants (ASVs) were generated through a SHA1-based hashing procedure applied to the nucleotide sequence which allows the same sequence to receive the same identified across datasets. Furthermore, taxonomic classifications were generated using reference databases, which included SILVA.

Prior to downstream analyses, several filtering steps were applied. Six inconsistent replicate samples (JMF-2602-01-0001A, JMF-2602-01-0021A, JMF-2602-01-0014A, JMF-2602-01-0007A, JMF-2602-01-0018A, and JMF-2602-01-0010A) were removed from the dataset. Additionally, contaminant ASVs identified through taxonomic inspection were excluded. Included in these excluded ASVs were *Pseudomonas (*contaminant in one culture) and *Dialister* (a contaminant from DNA extraction). A manual taxonomy correction was also

applied to ASV_n11_1uh, which was reassigned to the genus *Bacteroides* based on taxonomic evaluation.

This data was used for downstream analyses. The ASV count matrix, taxonomy table and sample metadata were the primary inputs for analyses in R (version 4.4.2). Data preprocessing involved the alignment of datasets by shared identifiers, where only intersecting samples were retained and combined into a single object using phyloseq package. Samples with 0 total reads were removed.

Raw counts reflecting the actual number of sequencing reads observed for each ASV was retained alpha diversity metrics. Next, relative abundance was generated by normalizing ASV counts within each sample by the total sequencing depth of that sample to remove sequencing-depth bias. This dataset was used for beta diversity metrics. Rarefaction was not performed but only used as quality control and visualization step to assess whether sequencing depth was sufficient. Instead, all subsequent analyses were performed on either raw count data or relative abundance data.

Alpha diversity was calculated using raw count data using the estimate richness function in phyloseq and Shannon diversity index were generated (Supplementary Figure S3). To identify if there are any differences between treatment groups, pairwise comparisons were performed using Wilcoxon rank-sum tests. Since multiple comparisons increase false positives, the resulting p-values were adjusted using Benjamini–Hochberg false discovery rate (FDR) correction.

The relative-abundance dataset was used for taxonomic composition. To this end, ASVs were collapsed at both the phylum and genus levels, and stacked bar plots were generated to visualize microbial community composition across treatments and biological replicates (Supplementary Figures S4–S5). Beta diversity was evaluated using Aitchison distance, calculated from centered log-ratio (CLR)-transformed count data with a pseudo count of one. Principal coordinates analysis (PCoA) was used to visualize differences in community composition among treatment groups. Statistical differences between groups were assessed using permutational multivariate analysis of variance (PERMANOVA). Pairwise

PERMANOVA analyses were performed and resulting p-values were adjusted using the Benjamini–Hochberg method. To verify that significant PERMANOVA results were not driven by unequal dispersion among groups, homogeneity of multivariate dispersions was assessed using PERMDISP (betadisper) with permutation testing.

To further investigate taxon level responses, enrichment factor (EF) analysis was performed. The mean relative abundance (%) was calculated across all groups. This results in values between -1 and 1, where positive values indicate enrichment relative to the control and negative values indicate depletion relative to the control. Statistical significance was determined using Wilcoxon rank-sum test and adjusted using the Benjamini–Hochberg method.

Chapter 4: Results

4.1 Dose-response effects of catecholamines are species specific and modulated by hemin availability

In the gut, bacteria are in direct contact with host-derived molecules such as hormones facilitating a bidirectional interaction with the host. Past studies have shown that host stress increases neuroendocrine hormones such as catecholamines could modulate bacterial physiology by influencing their growth, motility, and virulence. This in turn could disrupt the host's homeostasis. However, levels of catecholamines fluctuate widely in the gut and between each individual too. To this end, we explored how different concentrations of catecholamines affect bacterial growth. This approach allowed us to capture the full spectrum of bacterial growth dynamics from subtle to extreme conditions.

To assess dose-dependent effects of catecholamines on bacterial growth, 12 gut associated bacterial species were exposed to 6 different concentrations spanning from 10^{-6} to 10^{-1} mg/mL of adrenaline, dopamine, levodopa, and noradrenaline. Growth dynamics were quantified over 48h using Gaussian process regression and catecholamine-induced changes were expressed relative to the no-catecholamine (media only) control. These effects were assessed using the linear area under the curve (AUC; entire growth profile). The results showed highly species-specific growth patterns, with both growth promoting and inhibitory effects that were dependent on the hormone present, concentration, and the availability of hemin.

4.1.1 Catecholamines induce species-specific and concentration dependent responses in bacterial growth

Analysis of the AUC reveals a non-linear dose dependent and species-specific growth responses across all species and catecholamines (Figure 1). There is a clear distinction that some species accumulated growth with catecholamines while some species do not. Species specific responses were evident particularly in *Escherichia coli* and *Enterococcus*

mundtii with pronounced growth with all catecholamines across all concentrations. Moreover, in the *Bacteroides* genus, an intra-genus variability was observed. Out of the 4 *Bacteroides* species, only *Bacteroides finegoldii* showed robust and consistent increases in AUC across all 4 catecholamines. On the contrary, *Bacteroides thetaiotaomicron* showed no growth or minimal growth spanning all catecholamines and concentrations. *Bacteroides ovatus* and *Bacteroides caccae* also showed slight increases in growth.

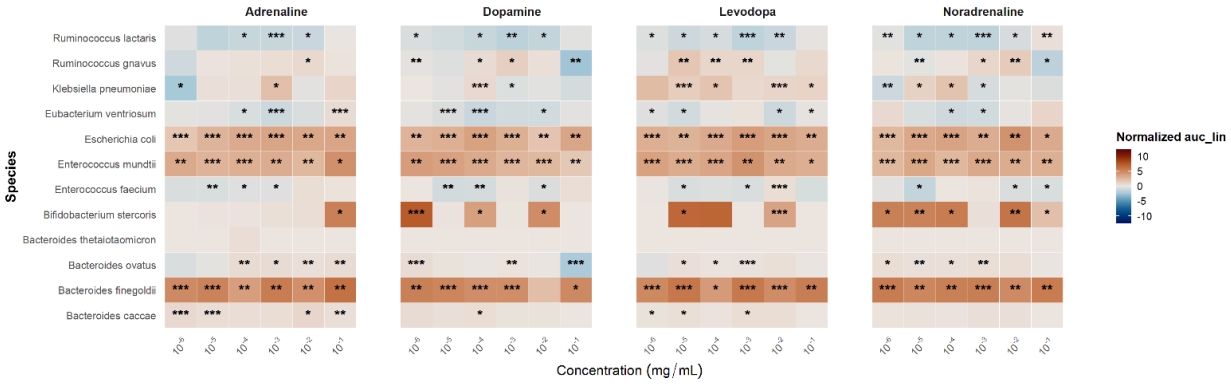


Figure 1: Heatmap of normalized AUC (area under the curve) without hemin supplementation
Heatmaps show control-normalized AUC for 12 bacterial species (y axis) exposed to increasing concentrations (x axis) of catecholamines. Values represent catecholamine induced changes relative to control without hormones. The color intensity corresponds to the magnitude and direction of growth responses and statistical significance is indicated by asterisks (($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

Moreover, a similar selective response can be seen in *Bifidobacterium stercoris* with dopamine, levodopa, and noradrenaline, where it shows more growth in comparison to adrenaline. However, the growth patterns of *B. stercoris* were more sporadic across concentrations with no clear pattern. In contrast to selective growth patterns, other taxa exhibited predominantly negative responses. *Ruminococcus gnavus, Ruminococcus lactaris, and Eubacterium ventriosum* showed consistent reductions in AUC across multiple catecholamines and concentrations, showing growth inhibition rather than stimulation.

When looking at the distribution of significant responses across all species and conditions, it can be said that the effects were also catecholamine and concentration specific (Supplementary Figure S6). This interesting variability can be seen in *Klebsiella pneumoniae*

with a rather mixed response depending on the catecholamine and concentration. Within the same hormone, the level of catecholamine had either growth stimulation or inhibition. For instance, in the lowest concentration of adrenaline (1 ng/ml) it showed growth inhibition but a significant increase in growth at 1 µg/mL. This is again visible with noradrenaline. Another species with a similar pattern was *R. lactaris*. It had decreased growth across all hormones and concentrations, except for an increase in growth at 100 µg/mL of noradrenaline. Some other species with similar catecholamine and concentration specific growth include *R. gnavus*, *E. faecium*, and *B. ovatus*.

4.1.2 The availability of hemin selectively modulates baseline growth in bacterial species

Having established the direct effects of catecholamines on the growth of these bacterial species, we next explored the role of hemin. Hemin is an iron-containing porphyrin that acts as a biologically available source of iron. This is crucial for bacterial cellular processes and the absence of iron can influence baseline bacterial growth even in the absence of catecholamines. Therefore, we first assessed the influence of hemin independent of catecholamines in the bacterial species as a baseline for interpreting subsequent catecholamine-induced effects with hemin supplementation (Figure 2).

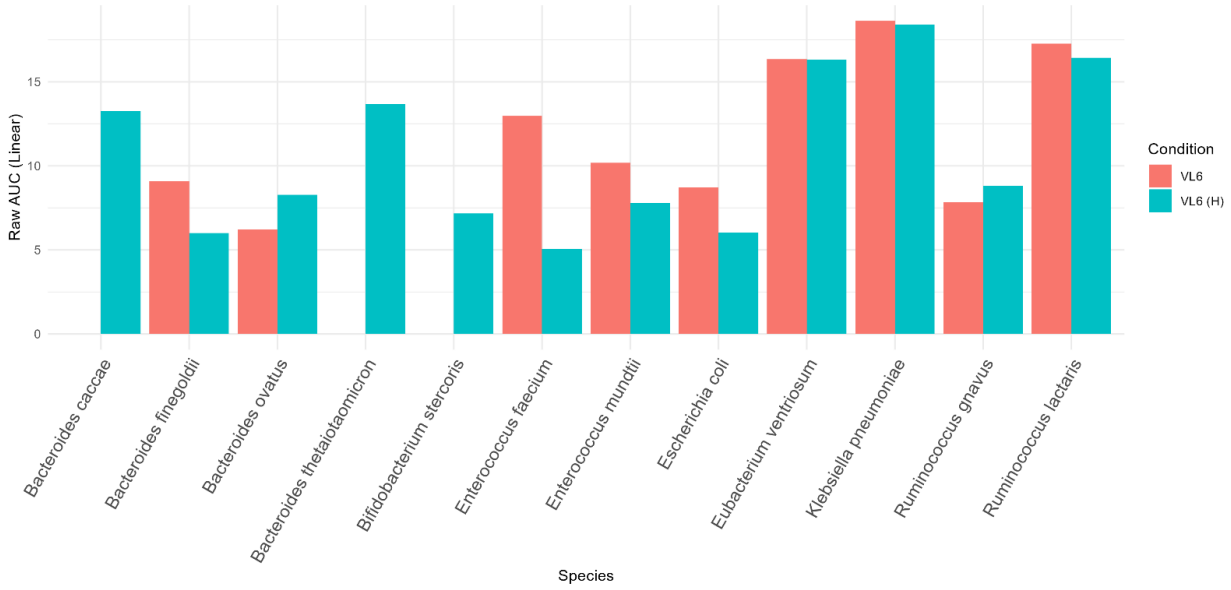


Figure 2: Effects of hemin on baseline bacterial growth in VL6 media
Bar plot showing the raw linear AUC values for the 12 bacterial species in control samples (media only) with hemin (turquoise) and without hemin (coral).

Looking at the total growth of the species in media with and without hemin, the direct effects of hemin are not uniform across species. 6 out of the 12 species showed clear increases in AUC when a form of iron is present. Interestingly, pathogenic, or opportunistic species such as *Escherichia coli*, *Enterococcus faecium*, *Enterococcus mundtii*, and *Klebsiella pneumoniae* generally showed robust baseline growth, but reduced growth with the supplementation of hemin. On the other hand, commensals like *Bacteroides caccae, B. ovatus,* and *B. thetaiotaomicron* had growth increases compared to their baseline growth without hemin.

4.1.3 Supplementation of hemin with catecholamines shifts growth responses

In order to determine growth dynamics with the availability of iron, the same dose-response analysis was conducted in catecholamine media supplemented with hemin. By supplementing hemin with catecholamines, we could next determine whether the previously observed effects are enhanced or diminished by answering the question whether iron availability changes how bacteria respond to catecholamines. Similarly, growth

responses were evaluated using the linear AUC (area under the curve) normalized to the control with hemin. Overall, with hemin supplementation, the magnitude of responses differed in comparison to cultures without hemin (Figure 3).

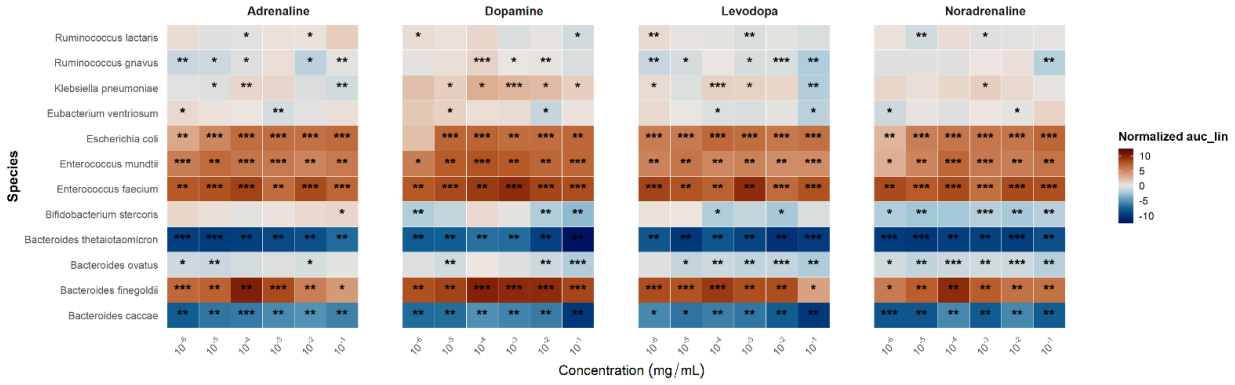


Figure 3: Catecholamine dose-response effects with hemin supplementation on bacterial growth
Faceted heatmaps show normalized linear area under the curve (AUC_lin) for 12 bacterial species exposed to increasing concentrations (10^{-6} to 10^{-1} mg/mL) of catecholamines (adrenaline, dopamine, levodopa and noradrenaline) in media supplemented with hemin. The color intensity reflects the magnitude and direction of growth responses while the asterisks indicated statistical significance relative to the control condition ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).*

A pronounced trend is the increase of growth in the facultative anaerobes and pathobionts like *Escherichia coli* and *Enterococcus* species (*E. mundtii* and *E. faecium*). These species already showed growth with several catecholamines but with hemin supplementation, this growth was enhanced and more uniform across concentrations. Dose-dependent responses seen earlier were not as prominent with the supplementation of hemin. With hemin, growth responses were more stable and sustained across concentrations.

While some species benefited from hemin supplementation, some commensals like the Bacteroides showed significant growth decreases or inhibition (Supplementary Figure 6). For example, *Bacteroides* ovatus in 1 µg/mL of adrenaline shifted the normalized AUC response from a slight increase in the absence of hemin (ΔAUC = 0.48) to pronounced inhibition with hemin supplementation (ΔAUC = -0.45). Again, the intra-genus variability was observed

when looking at *Bacteroides finegoldii* that continues to grow with hemin supplementation in levodopa (No hemin ΔAUC = 5.62; Hemin ΔAUC = 7.83).

To complement the concentration-focused analyses, catecholamine induced growth results were additionally summarized across all the doses for each hormone. For this analysis, normalized AUC values were pooled across all concentrations for each species in a given hormone to compare the effects of hemin supplementation (Supplementary Figure S7). When growth responses were collapsed across concentrations, there was a consistency with the dose-response analyses showing highly species-specific effects. With the presence of hemin, an increased spread and variability was observed for all 4 catecholamines. However, collectively, these shifts were not statistically significant (adrenaline $p = 0.886$; dopamine $p = 0.889$; levodopa $p = 0.61$; noradrenaline $p = 0.782$). This indicates a substantial inter-species variability rather than a uniform global effect of hemin. This heterogeneity mirrors the dose-dependent patterns observed earlier and reinforces that hemin availability can shift overall growth patterns, however the magnitude and direction of catecholamine effects are primarily determined at the species level.

4.2 Catecholamine-induced bacterial growth dynamics are modulated by the substrate present

Having established that catecholamine effects on bacterial growth are species specific in the dose-response assay, we next extended the analysis to dietary polymers. To this end, we compared growth in polymer-only media to growth in the same polymer media supplemented with catecholamines for 11 species out of the 12. As polymer degradation or utilization was not measured directly, we interpreted differences between polymer-only and hormone supplemented polymer media as polymer utilization. These results were quantified using control-normalized auc_lin values, which capture the total growth profile over 48 hours.

4.2.1 Baseline bacterial growth differs across polymer substrates

Prior to determining catecholamine effects on bacterial growth with polymer substrates, it was first necessary to establish the baseline growth in polymer-containing media. This baseline growth varied considerably between both species and the polymer conditions (Figure 4). Across the 6 polymers, the highest auc_lin values were observed in inulin, pullulan and pectin containing media. Within these conditions, the high auc_lin values were mostly associated with *Ruminococcus lactaris* and *Klebsiella pneumoniae*. In particular, *R. lactaris* showed the greatest growth in pullulan and pectin-containing media, while *K. pneumoniae* displayed high growth in inulin, pullulan, and pectin.

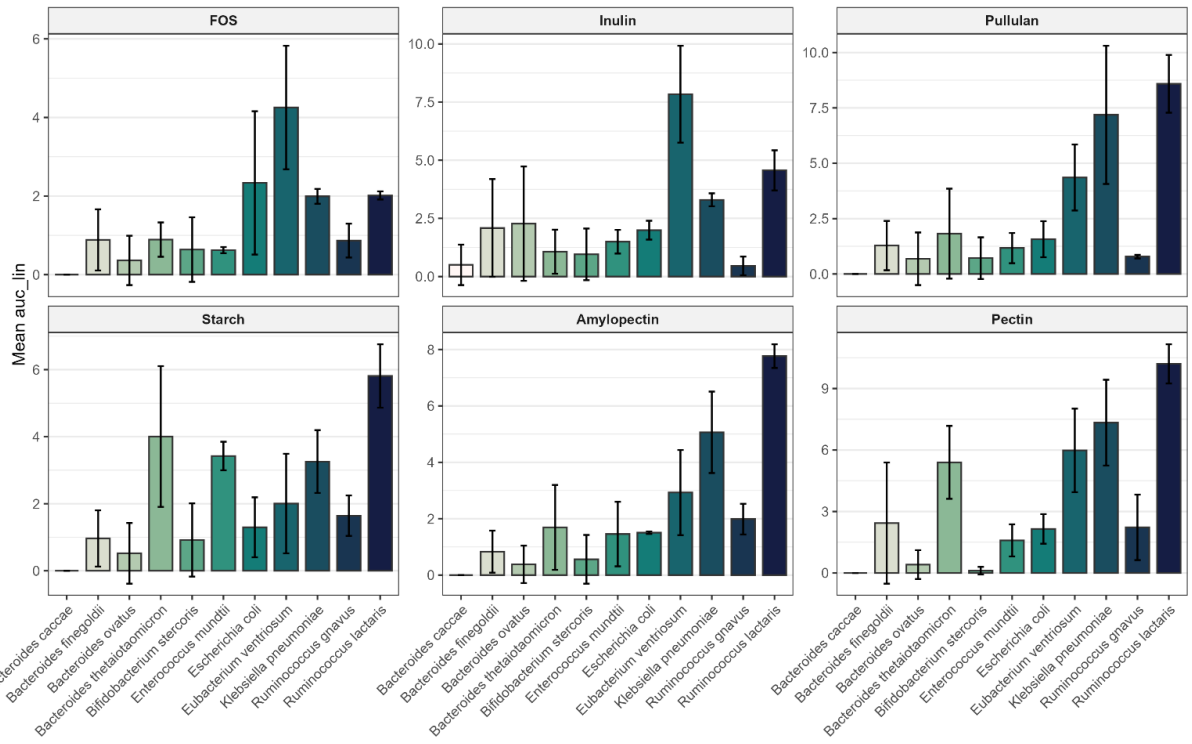


Figure 4: Growth of bacterial species in baseline polymer media
Bar plot shows the mean area under the curve (auc_lin) for each species in polymer containing media. Growth was measured across six polymers: fructo-oligosaccharides (FOS), inulin, pullulan, starch, amylopectin, and pectin. Each facet corresponds to a single polymer, and the bars represent each species (x axis). Error bars indicate variability (mean ± SD).

On the other hand, growth in FOS containing media was generally lower, although some species like *Eubacterium ventriosum* maintained relatively high growth in comparison to the other species and was also consistent across all polymer conditions. Another consistent responder across all polymers was *Bacteroides caccae* that exhibited consistently low growth with auc_lin values remaining below 1.

Despite these consistent growth patterns, some species also have varying responses. This variation was also observed within similar species and between species. Within the *Bacteroides* genus, for example, *B. thetaiotaomicron* displayed higher growth in starch- and pectin-containing media, whereas *B. ovatus* and *B. caccae* generally exhibited lower growth across most polymer conditions. Similarly, species such as *Escherichia coli* showed growth profiles that varied across the polymer conditions, with lower auc_lin values in starch and higher growth values in FOS-containing media. Overall, the results demonstrate distinct species-specific growth profiles across the six polymer conditions.

4.2.2 Distinct catecholamine response profiles emerge across polymer substrates

Given these differences in baseline growth, it highlights the importance of accounting for these inherent growth capabilities when assessing catecholamine-mediated effects. Therefore, the next approach was to determine if catecholamine supplementation would alter these baseline growth profiles. Growth responses were quantified as control-normalized auc_lin values relative to the corresponding polymer-only controls (Figure 5). These growth responses are also reported below as fold changes, calculated as the mean auc_lin in catecholamine-supplemented media divided by the mean auc_lin of the corresponding polymer-only control.

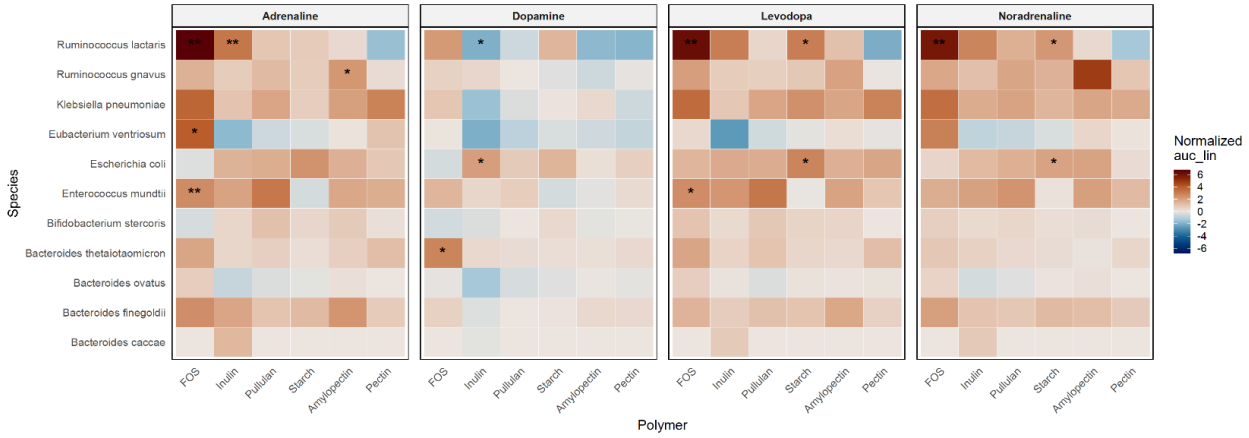


Figure 5: Effects of catecholamines on bacterial growth with polymer availability
Faceted heatmap displaying the normalized auc_lin of 11 bacterial species (y axis) cultured on 6 polymer substrates (x axis) ordered by increasing structural complexity: FOS, Inulin, Pullulan, Starch, Amylopectin, and Pectin. The polymer media was supplemented by the catecholamines adrenaline, dopamine, levodopa, and noradrenaline. Positive values (orange/red) indicate enhanced growth compared to polymer-only media controls, while negative values (green) indicate growth inhibition. Asterisks denote statistical significance ($p < 0.05$, ** $p < 0.01$).*

Generally, catecholamine supplementation resulted in predominantly positive growth, however, only few of these growth responses were significant in comparison to polymer only media. Adrenaline produced the most significant growth responses, followed by levodopa. Most of these significant effects were observed in FOS-containing media. Among the species, *Ruminococcus lactaris* emerged as the most responsive species. In FOS-containing media, supplementation with adrenaline, levodopa, and noradrenaline increased growth approximately 4.4-fold, 4.1-fold, and 3.9-fold relative to the polymer-only control, respectively. Additionally, growth was consistently increased in starch containing media with levodopa and noradrenaline, with 1.5-fold and 1.4-fold increases, respectively. *Enterococcus mundtii* exhibited the largest relative growth responses. In FOS-containing media, adrenaline and levodopa supplementation resulted in approximately 5.1-fold and 5.0-fold increases in growth relative to the control.

Although dopamine increased growth in some species, it was also the catecholamine with the most reduced growth relative to polymer only controls. An interesting pattern observed was the opposing effects of adrenaline and dopamine with inulin containing media for *R.*

lactaris. While adrenaline increased growth by 1.7-fold, dopamine significantly reduced to 0.55-fold of the control (≈45% reduction). This pattern can also be seen with *Klebsiella pneumoniae* in inulin containing media. Even though it was not significant, adrenaline, levodopa and noradrenaline produced slightly more growth, while dopamine had negative growth for *K. pneumoniae*. Similarly, *Ruminococcus gnavus* had negative growth with dopamine in amylopectin containing media but positive growth with the other catecholamines. Together, these results show a hormone and species-specific growth pattern under polymer-based conditions.

4.2.3 Genus-level responses reveal broader substrate-dependent enrichment patterns

The findings thus far has identified species-specific growth responses to catecholamine supplementation. However, this variability could also reveal a broader genus level pattern. Therefore, an enrichment factor analysis was used to determine whether these responses reflected broader genus-level trends and whether such responses were influenced by the available substrate (Figure 6). These results show that catecholamine effects on bacterial growth were highly dependent on substrate.

The type of substrate was a major determinant of catecholamine-mediated enrichment. Among the polymers, FOS was associated with the most consistent positive enrichment of multiple genera including *Bacteroides*, *Enterococcus*, *Klebsiella*, and *Ruminococcus*. Whereas pectin was associated with the fewest catecholamine-mediated enrichments. Neutral patterns were observed for inulin, pullulan, starch, and amylopectin, where enrichment varied according to both the genus and catecholamine present.

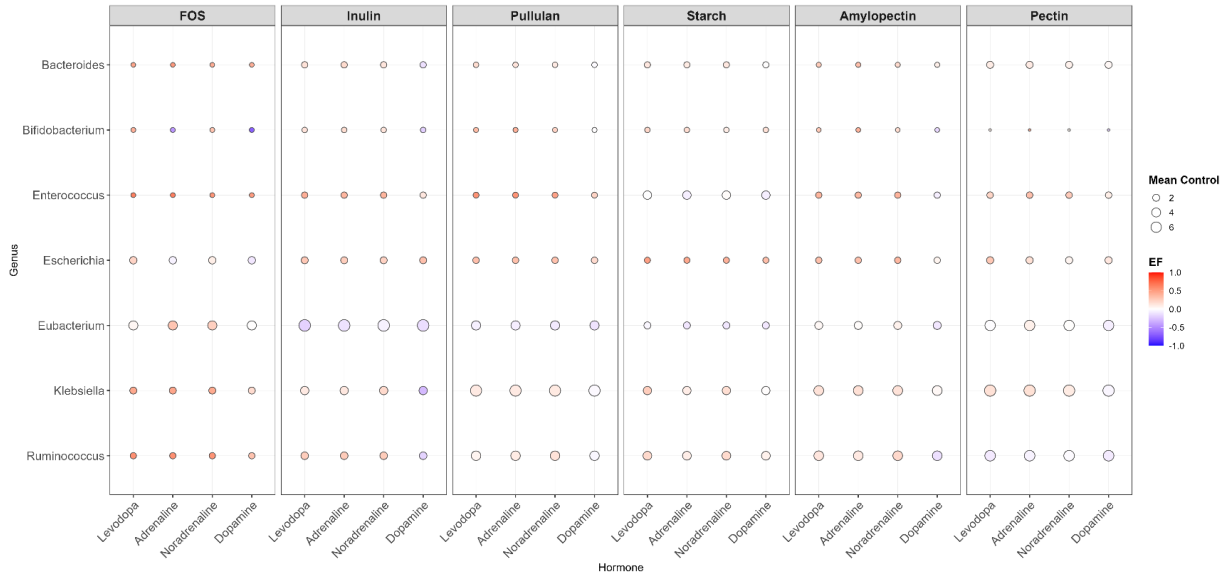


Figure 6: Enrichment analysis of bacteria with catecholamines and polymers
Faceted bubble plot showing the effects of catecholamines supplementation on bacterial growth in polymer containing media. Bubble size represents the mean abundance in the control group, while bubble color indicates the enrichment factor (EF), with red denoting positive effects and blue denoting negative effects relative to the control. The x-axis shows the catecholamines (levodopa, adrenaline, noradrenaline, and dopamine), and the y-axis shows the bacterial genera.

Amongst genera that showed consistent responses, *Enterococcus* and *Escherichia* were the most consistently enriched genera. They were positively enriched across most polymer-hormone combinations. In contrast, *Eubacterium* frequently had negative enrichments regardless of catecholamine or polymer present. Other genera showed context dependent responses. *Ruminococcus*, *Bacteroides*, and *Bifidobacterium* exhibited varying enrichment patterns that differed according to substrate type, with positive responses observed under some conditions and negative responses under others. Another notable pattern was the distinct behavior of dopamine relative to the other catecholamines. Across multiple genera and polymers, dopamine frequently produced negative enrichments than adrenaline, levodopa, or noradrenaline. This divergence is clearly seen in inulin and pullulan-containing media, where dopamine was associated with reduced enrichment across most genera. This genus-level enrichment analysis demonstrated that catecholamine-mediated growth responses are shaped by strong interactions between the type of catecholamine and substrate available.

4.2.4 Shared CAZyme profiles among responsive species suggest potential enzyme-mediated catecholamine effects

The genus- level enrichment analysis showed catecholamine mediated growth responses with polymers were influenced by the type of polymer and also the type of catecholamine present. However, several significant responses were repeatedly observed among species in the same polymer-catecholamine conditions. This raised the question of whether these species shared a common functional profile that could contribute to the similar growth responses in polymers.

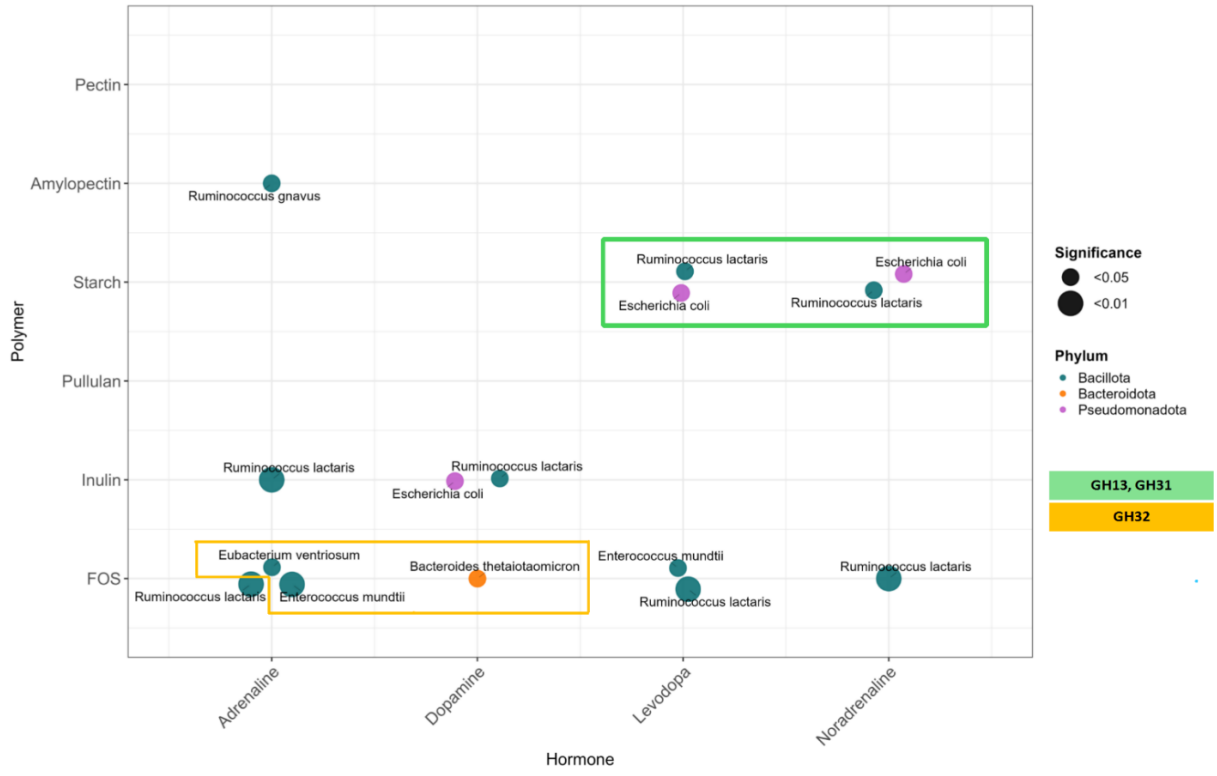


Figure 7: Shared CAZyme families among bacterial species exhibiting significant responses in catecholamine-polymer media
Bubble plot showing bacterial species that exhibited significant growth responses to catecholamine supplementation under polymer-containing conditions. Bubble color indicates phylum, while bubble size denotes statistical significance. Green boxes highlight species responding that share glycoside hydrolase families (GH13 and GH31); orange boxes for GH32 enzymes.

In particular, if catecholamines influence bacterial growth through mechanisms linked to nutrient acquisition, then species enriched under the same conditions may share common

carbohydrate-active enzymes (CAZymes). To investigate if similar enzyme profiles existed, the CAZyme repertoires of species exhibiting significant growth responses were examined, focusing on glycoside hydrolase (GH) families associated with the degradation on the corresponding polymer substrates (Figure 7). Distinct clusters of species enriched in polymer-catecholamine with significant growth responses shared CAZyme profiles. In starch-containing media, both *Ruminococcus lactaris* and *Escherichia coli* showed significant positive growth responses to levodopa and noradrenaline supplementation. The genomes of these species encoded glycoside hydrolase families GH13 and GH31, which are commonly associated with starch degradation.

A second observation emerged in FOS-containing media, where *Ruminococcus lactaris*, *Enterococcus mundtii*, and *Eubacterium ventriosum* showed positive significant responses with the supplementation of adrenaline and *Bacteroides thetaiotaomicron* with dopamine. Interestingly, although *Ruminococcus lactaris* showed significantly positive growth in FOS media, its annotated GH profile did not indicate the presence of enzymes associated with FOS degradation. Nevertheless, *Enterococcus mundtii*, *Eubacterium ventriosum* and *Bacteroides thetaiotaomicron* shared the GH32 family, which is associated with the degradation of fructans and fructooligosaccharides. These results show that species displaying similar growth responses within the same polymer-catecholamine conditions frequently possessed GH families associated with degradation of the corresponding substrate. Although this analysis does not confirm direct regulation or modulation of CAZymes by catecholamines, or the expression and activity of enzymes, the observed overlap in enzyme profiles within a given polymer environment provides evidence that species responding similarly often possess comparable carbohydrate degradation capabilities.

4.3 Catecholamine exposure influences community composition

So far, the experiments conducted were in monocultures that fail to capture community level interactions. The effects of catecholamine observed were driven by species identity and further modulated by the presence of iron and polymers. These analyses Within the gut,

bacteria form complex communities where interactions between species can influence individual responses. Therefore, to understand if observed species-specific responses translate on a community level, a synthetic microbial community (SynCom) was created with the same 12 species. Passaging was performed over 5 consecutive days and the samples from the final day were analyzed using 16S rRNA sequencing. This approach helps determine if catecholamine exposure influences overall community diversity and composition.

4.3.1 Exposure to catecholamines changed the relative abundances of species within the community

At the phylum level, the SynCom communities were primarily composed of *Bacillota*, *Bacteroidota*, and *Pseudomonadota* across all treatment conditions (Supplementary Figure S5). On the genus level, the communities were dominated by *Bacteroides (~31–37%)*, *Enterococcus (~27–31%)*, and *Escherichia-Shigella (~13–15%)*. However, the relative abundance profiles between the catecholamine treatments differed for each genus (Figure 8). *Bacteroides* had their highest relative abundance in dopamine treated samples at 37%, followed by adrenaline and noradrenaline at 35%, levodopa at 32% and the control at 31%. In comparison, *Enterococcus* showed an inverse effect with the highest relative abundance in the control and levodopa-treated samples (~31%), but lower relative abundances in adrenaline (~29%), noradrenaline (~30%), and dopamine (~27%). *Escherichia-Shigella* remained stable across catecholamine treatments with moderate variations between ~13-15% relative abundances. Species that were low across all conditions were *Ruminococcus* and *Bifidobacterium*. Although one *Eubacterium* species was included in the SynCom, the species was not detected in any of 16S rRNA sequencing datasets.

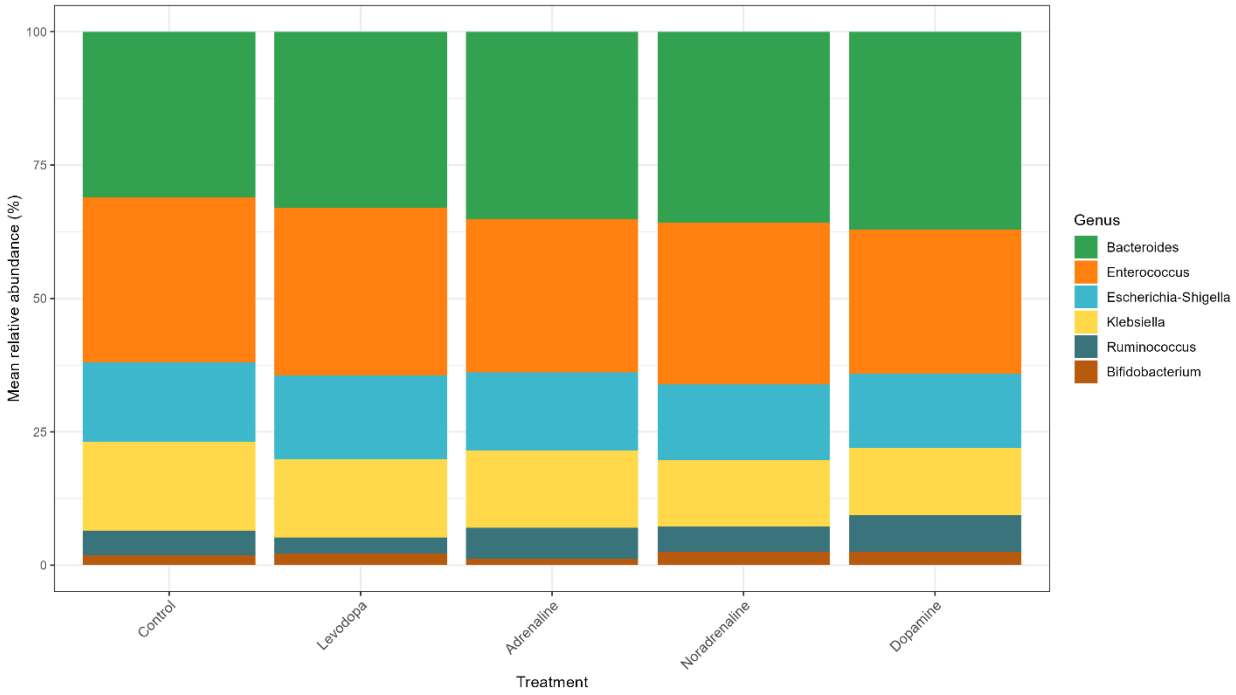


Figure 8: Relative abundances (%) of the species within the synthetic communities across the catecholamine treatments

The x-axis represents the treatments while the y-axis indicates the mean relative abundance (%). The colors represent the genus of bacteria as indicated in the legend. The stacked bars represents the proportional contribution of the detected species to the total community composition within each condition.

Building on these genus level observations, some trends in relative genus abundance was observed in the communities. The only genus that increased their relative abundance across all catecholamines were the *Bacteroides*, while *Klebsiella* abundance decreased across all catecholamine-treatments relative to the control. Therefore, it can be said that the *Bacteroides* and *Klebsiella* had inverse responses. Another pattern observed was the similarity in relative abundances for *Enterococcus* and *Escherichia-Shigella*. Both had increasing abundance with levodopa but decreased across adrenaline, noradrenaline, and dopamine communities. *Ruminococcus* and *Bifidobacterium* had the most distinct profiles with no clear pattern or similarity to other species while also exhibiting the highest differences in relative abundances observed. In *Ruminococcus,* levodopa reduced the relative abundance from 4.6% in the control community to 3.0% corresponding to a 34.2% reduction in relative abundance, while increasing to 5.8% and 6.9% in adrenaline- and

dopamine-treated communities, representing increases of 26.0% and 49.0%, respectively. *Bifidobacterium* had a reduction of its relative abundance with adrenaline (−36.0%) but showed substantial increases under dopamine (+35.4%), noradrenaline (+34.8%) treatments, and levodopa (+17.0%). Collectively, these results show that catecholamine exposure altered the relative abundances of multiple genera in the synthetic community.

4.3.2 Beta-diversity analysis reveals treatment-dependent shifts in SynCom composition

So far, the genus level shifts observed could also contribute to differences between the catecholamine treated communities. To further understand if the communities themselves were compositionally different, beta diversity analysis was performed using Aitchison distance on centered log ratio (CLR) -transformed 16S rRNA profiles.

Principal coordinates analysis (PCoA) indicates clear separation of treatment groups, primarily along PCoA1 (Figure 9). PCoA1 explained 62.1% of the total variance, while PCoA2 explained 15.1% of the variance. This indicates that the biggest variation between communities was observed on PCoA1. Some distinct clusters were also noted between treatment groups. The control samples had clusters on the negative axis of PCoA1. These samples had consistent composition between their replicates resulting in tight clusters. Dopamine treated samples also clustered on the negative side of PCoA1 similarly to the control and also had consistency between the replicates. Although levodopa acts as a biochemical precursor for dopamine, dopamine treated communities display contrasting clustering patterns. Although, adrenaline and noradrenaline had similar genus level relative abundances observed earlier, they had the biggest dispersion in community composition between replicates with spread out data points.

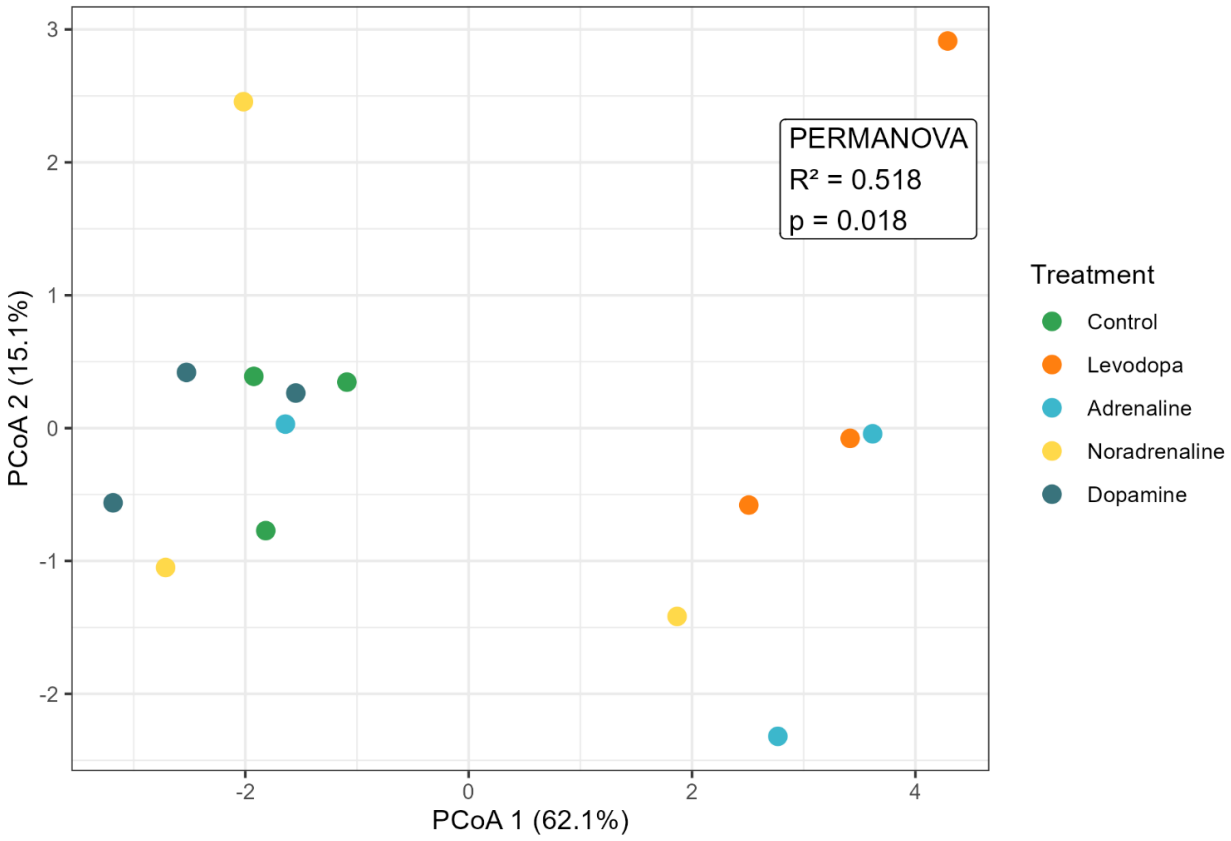


Figure 9: Principal coordinates analysis (PCoA) plot based on synthetic community composition across the catecholamines

The axes represent the first two principal coordinates derived from the Aitchison distance matrix, where PCoA1 explained 62.1% of the total variation in community composition and PCoA2 explained 15.1%. Each point represents an individual sample from the corresponding treatment group that is color coded. Furthermore, PERMANOVA analysis showed significant differences between the treatment groups (R^2 = 0.518, p = 0.018), where the R^2 value indicates that 51.8% of the variation in community composition was explained by treatment, while the p-value indicates that these differences were statistically significant.

Additionally, PERMANOVA analysis was carried out on the Aitchison distance matrix to evaluate if these differences were statistcally significant. The results show that catecholamine treatment signficantly influenced community composition of the synthetic community (R^2 = 0.518, p = 0.018) resulting in 51.8% of the observed variation and this effect was statistically significant. Since significant PERMANOVA results can also arise from differences in unequal spread within groups, multivariate dispersion was assessed using

PERMDISP. No significant differences in dispersion were observed between treatment groups (F = 3.245, p = 0.058; Supplementary Figure S8).

4.3.2 Differentially enriched genera are associated with the catecholamine induced community shifts

Given that the beta diversity analysis showed catecholamine induced community composition shifts, it remains unclear which taxa or species drive these changes. To address this, enrichment analysis was performed at the genus level to determine catecholamine induced shifts at the genus level (Figure 10).

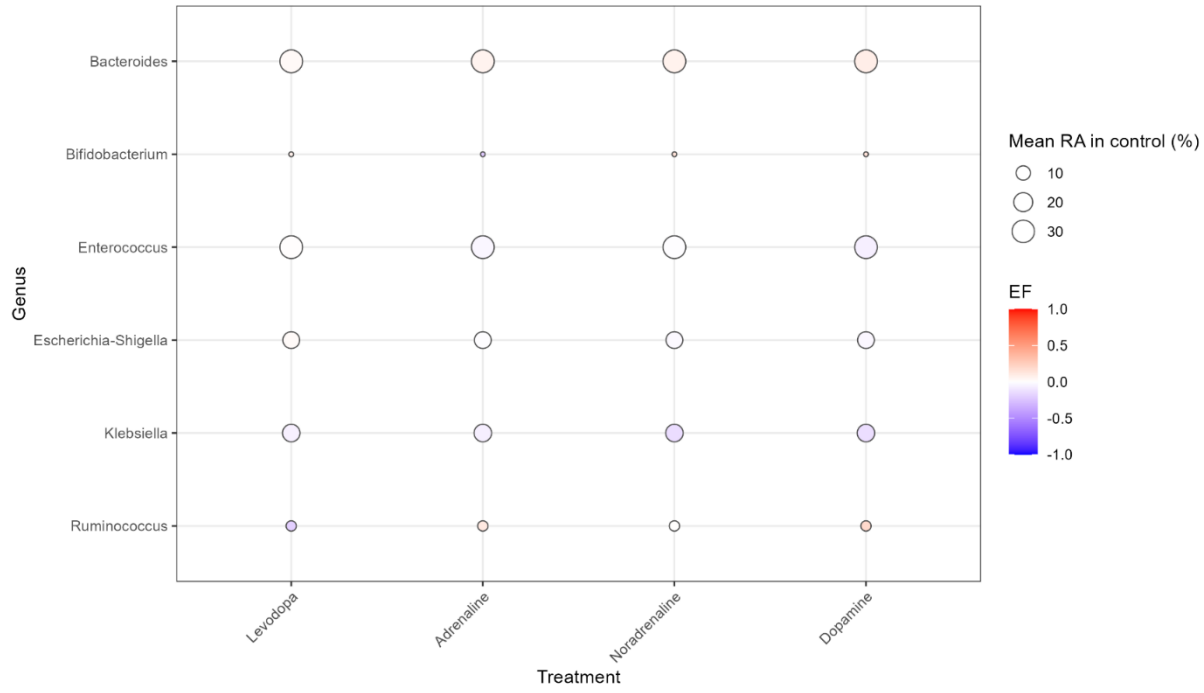


***Figure 10*: Genus level enrichments in synthetic communities across catecholamines**
Bubble plot representing changes in genus-level relative abundance. The color represents the enrichment factor (EF) where red = enrichment and blue = depletion. The size of the bubble reflects the relative abundance in control samples where larger bubble size = more abundant in control and smaller bubble = less abundant in control.

Consistent with the previously observed relative abundance profiles, the genus level enrichment analysis provided a measure of the direction and magnitude of responses relative to the control. Overall enrichment factors were small, ranging from −0.219 to +0.196.

This is also consistent with the community composition data that showed modest changes in relative abundance across the treatment groups.

Despite the small enrichment magnitudes, some distinct patterns were observed. While most genera showed consistent enrichment profiles like the *Bacteroides* and *Klebsiella,* the most distinct enrichment patterns were observed for *Ruminococcus* and *Bifidobacterium*. Unlike the genera that were more abundant, these two species that were low in abundance showed treatment specific enrichment profiles with contrasting responses dependent on the catecholamine present (Supplementary Table S4).

Amongst the positive enrichments, the strongest enrichment was observed for *Ruminococcus* under dopamine treatment (EF = 0.197). The second largest enrichment was observed for *Bifidobacterium* under dopamine (EF = 0.150), followed by noradrenaline treatment (EF = 0.148). Positive enrichment was also seen for *Ruminococcus* under adrenaline and noradrenaline treatments, while *Bifidobacterium* was enriched with levodopa treatment. In contrast, these two species also had the strongest depletion responses. The strongest depletion was observed for *Bifidobacterium* under adrenaline treatment (EF = -0.220), representing the lowest enrichment factor identified across all genera and treatments. Similarly, *Ruminococcus* was depleted under levodopa treatment (EF = -0.206), corresponding to the second-lowest enrichment factor observed. Together these results identify *Ruminococcus* and *Bifidobacterium* as the genera exhibiting the most treatment-dependent responses within the synthetic communities.

Overall, findings from the dose-response assay, hemin, and polymer supplementation assays, and syncom analyses revealed that catecholamine effects cannot be explained by a single universal pattern. Instead, they were dependent on multiple factors such as the species identity, the type and concentration of catecholamine, the supplementation of hemin and polymer. The magnitude and direction of these effects differed between each of these contexts. Moreover, these individual responses in monocultures were not consistently translated at the community level, highlighting the function of catecholamines as ecological modulators shaping bacterial responses.

Chapter 5: Discussion

This study demonstrates that catecholamines exert complex and highly context-dependent effects on gut bacterial growth and community composition. Across the bacterial species examined, growth responses to the catecholamines varied depending on species identity, hormone concentration, iron, and polymer presence. Overall, these findings support the concept of microbial endocrinology, whereby host derived neuroendocrine signals influence bacterial physiology (Lyte and Ernst 1992), while also highlighting that such interactions are not universal across species. Instead, catecholamine-mediated responses are regulated by species-specific physiological traits and environmental conditions, further suggesting that the impact of host stress hormones on the gut microbiota is shaped by the context in which these interactions occur.

One of the most consistent findings was the species-specificity of catecholamine responses. While species such as *Escherichia coli* and *Enterococcus mundtii* exhibited pronounced growth across multiple catecholamines and concentrations, other species like *Ruminococcus gnavus*, *Ruminococcus lactaris* and *Eubacterium ventriosum* showed reduced growth. Looking at the members of the *Bacteroides* genus, we see diverging responses even though they are closely related species. This observation is consistent with previous findings suggesting that phylogenetic similarity alone cannot effectively predict functional behavior. A similar observation made by Lozupone et al. (2012), shows that species-specific regulatory networks and metabolic capabilities govern their responses to stimuli rather than taxonomic similarity.

This variability in responses may also reflect how individual species perceive and process catecholamines. In the emerging field of microbial endocrinology, it is evident that catecholamines can function as inter-kingdom signaling molecules where it could enhance growth in pathogenic species or virulence in species like *Escherichia coli* (Lyte and Ernst, 1992; Lyte and Ernst, 1993; Clarke et al., 2006). In some bacterial species, particularly members of the *Enterobacteriaceae*, adrenaline and noradrenaline can be detected by the sensor kinase QseC, triggering signaling pathways involved in motility and virulence gene

expression (Clarke et al., 2006; Niu et al., 2023). On the other hand, species like *Ruminococcus* and *Eubacterium ventriosum* showed negative growth. One possibility is that the capacity to detect and respond to catecholamines in these species may vary substantially and therefore they cannot exploit catecholamines as growth promoting signals. Another possibility is that catecholamines can also impose metabolic stress through oxidation reactions that produce reactive oxygen species and quinone derivatives that can affect microbial physiology (Oleskin et al., 2021; Prah & Mavri, 2024). While the precise mechanisms underlying these responses remain unclear in our findings, the observed variability suggests that bacterial responses to catecholamines are shaped by species-specific physiological characteristics rather than by a single sensing pathway.

This complexity became further evident when considering the influence of catecholamine concentration, which altered both the magnitude and direction of bacterial growth responses. Rather than producing consistent increases or decreases in growth, catecholamine responses were frequently non-linear across the concentration range tested. This is noticeably clear in the growth patterns of *Klebsiella pneumoniae*, which displayed growth inhibition at lower adrenaline and noradrenaline concentrations but significant growth enhancement at intermediate concentrations. Similar concentration-dependent responses have been reported previously. Using a luminescent *E. coli* biosensor, Oleskin et al. (2017) observed enhanced bacterial bioluminescence at lower dopamine concentrations and inhibition at higher concentrations. Although this kind of bioluminescence reflects metabolic activity rather than bacterial growth, these findings nevertheless support the notion that catecholamines can exert biphasic effects on bacterial physiology depending on concentration.

In this study, such biphasic behavior may arise because catecholamines can serve multiple functions within bacterial systems, including signaling, nutrient acquisition, and modulation of cellular metabolism (Karavolos & Khan, 2013). At lower concentrations, catecholamines may activate favorable signaling pathways. However, at higher concentrations, beneficial effects might be reversed by metabolic stress or toxicity, associated with catecholamine oxidation and the generation of reactive intermediates (Oleskin et al., 2021). Although the

underlying mechanisms were not investigated in this study, the observed dose dependence highlights the importance of considering hormone concentration when evaluating host-microbe interactions.

While catecholamine concentration influenced the magnitude and direction of bacterial growth responses, it was not the only factor contributing to the observed variability. The surrounding growth factors such as hemin also played a significant role in modulating growth responses. The effects of hemin were highly species-specific, with some species benefiting while some showed reduced growth. This species-specific variability is consistent with previous observations that bacterial taxa differ in their capacity to acquire and utilize hemin-derived iron (Celis et al., 2023). Therefore, heme availability can selectively alter the growth of bacteria.

An unexpected observation was that at the baseline growth, hemin supplementation altered many growth patterns. However, these patterns were not consistent with the responses to catecholamines under hemin supplemented conditions. Species from the *Bacteroides* genus benefited from hemin supplementation in the media alone but frequently exhibited reduced growth with hemin supplemented catecholamines. On the contrary, species such as *Escherichia coli* and *Enterococcus* showed little benefit with hemin at their baseline growth but had the strongest catecholamine-associated growth responses with hemin supplementation. The disconnect between baseline hemin responses and catecholamine associated growth suggests that catecholamine signaling is a major determinant beyond iron acquisition alone. While hemin primarily influences bacterial growth through its role as an iron source, catecholamines appear to exert additional effects through bacterial signaling pathways. Previous studies have shown that catecholamines can regulate gene expression independently of iron acquisition, influencing processes such as stress adaptation and nutrient uptake (Clarke et al., 2006; Karavolos & Khan, 2013). Therefore, species such as *Escherichia coli* and *Enterococcus* may derive greater benefit from catecholamine-mediated signaling than from hemin supplementation alone, whereas members of the *Bacteroides* genus may respond positively to hemin availability but not for catecholamines.

An additional level of complexity was introduced by examining microbial growth on dietary polymers. These structurally diverse substrates provided a broader framework for assessing how nutrient availability influences catecholamine-mediated growth responses. One of the primary functions of gut bacteria is the metabolism of dietary carbohydrates and the production of short-chain fatty acids (SCFAs) (Hammouda et al., 2023). The ability to utilize these substrates is determined by carbohydrate-active enzymes (CAZymes), which vary between bacterial species (Wardman et al., 2022). However, substrate utilization is influenced not only by bacterial enzymatic capacity but also by the structural characteristics of the polymer itself. Differences in glycosidic linkages, branching patterns, and accessibility affect how readily a substrate can be degraded and utilized, resulting in varying degrees of specialization among bacterial taxa (Martens et al., 2014). Consequently, bacterial growth responses are expected to differ across polymer environments, reflecting the interaction between substrate structure and species-specific metabolic capabilities.

Notably, catecholamine-associated growth enhancement was most pronounced in FOS-containing media, whereas comparatively weaker responses were observed in more structurally complex substrates such as pectin. One explanation is that simpler substrates such as FOS are more easily accessible and utilized (Hamaker & Tuncil, 2014), as they require less extensive enzymatic processing, resulting in greater baseline growth and making any growth-promoting effects of catecholamines more apparent. In contrast, the degradation of complex polymers such as pectin requires coordinated activity of multiple enzymes and may therefore impose additional metabolic constraints that limit bacterial growth irrespective of catecholamine exposure.

Furthermore, the CAZyme repertoires of the species were predicted using a bioinformatic pipeline and examined to compare with their growth responses across the tested polymers. The predicted CAZyme repertoires did not always translate to growth responses. Some species possessing the enzyme capacities for a polymer showed only modest growth responses. This data shows that the presence of a gene may not be sufficient to predict growth outcomes. Nevertheless, some species showing significant growth enhancements in particular polymers possessed enzyme families associated with degradation of those

polymers. For instance, significantly responsive species in starch containing media, commonly possessed members of the GH13 and GH31 family and those in FOS media containing GH32 enzymes. These observations support previous findings that CAZyme repertoires strongly influence substrate specialization explaining species-specific growth differences across different polymers (Martens et al., 2014). While CAZymes determine access to polymers, species with similar predicted carbohydrate utilization capacities often showed varying responses based on the catecholamine. For instance, although *Bacteroides thetaiotaomicron, Enterococcus mundtii and Eubacterium ventriosum* contained the GH32 enzyme for FOS degradation, their responses varied with the type of catecholamine. Although gene expression was not measured in this study, the observed differences between species with comparable CAZyme profiles raise the possibility that catecholamines interact with these regulatory systems, thereby modifying growth outcomes independently of substrate degradation capacity.

Comparison of responses across these two different experimental contexts showed that the responses to catecholamines were not a fixed trait of individual species. Instead, the direction and magnitude of response often depended on the environmental context in which bacteria were assessed. This was especially clear for *Bacteroides* and *Ruminococcus*. In the dose response assay, both genera typically showed negative enrichments to catecholamine exposure. However, in the polymer experiments, these responses shifted towards positive enrichment, especially under substrates that were easily accessible. The change in response direction suggests that context can significantly change how bacteria interact with host neurochemicals. Rather than acting on their own, catecholamine effects seem to depend on the metabolic state of the cell. Transcriptional profiling has shown that catecholamine exposure can alter the expression of genes (Dowd, 2007; Boujnane et al., 2024). Collectively, these findings suggest that catecholamine responses emerge from interactions between multiple regulatory networks. The same neurochemical may therefore produce contrasting outcomes under different environmental conditions, highlighting the highly context-dependent nature of microbial endocrinological interactions.

While the monoculture experiments showed species-specific responses to catecholamines, bacteria within the gastrointestinal tract rarely exist in isolation. Instead, they function within complex communities where growth is not only affected by intrinsic characteristics but also by community interactions. Therefore, we also addressed the question of whether species-specific responses translate into changes in community composition. Amplicon sequencing revealed that catecholamine effects were not limited to individual species but also influenced the structure of the synthetic community. Although overall diversity remained stable, beta diversity analysis revealed significant differences between catecholamine conditions. This distinction was interesting as it showed catecholamine effects altered the relative abundance of species within the community. Principal coordinate analysis showed treatment-dependent clustering patterns, while PERMANOVA analysis confirmed that catecholamine treatment explained a considerable proportion of the variation in community composition ($R^2 = 0.518$, $p = 0.018$), demonstrating that host neurochemicals can influence microbial community structure even in simplified *in vitro* systems. Furthermore, the absence of significant differences in multivariate dispersion suggests that these compositional shifts reflect treatment effects rather than variation in community stability among replicates.

Interestingly, several patterns observed at the community level differed from those seen in monoculture. For instance, species such as *Bacteroides* showed consistent enrichment across treatments, whereas others, like *Klebsiella*, decreased in relative abundance. *Escherichia coli* and *Enterococcus* species frequently exhibited strong positive growth responses during the dose-response experiments, particularly in the presence of hemin. However, these responses did not translate into relative abundance within the synthetic communities. In contrast, *Ruminococcus* and *Bifidobacterium*, which often showed weaker or inconsistent responses in monoculture, displayed some of the most pronounced treatment-specific responses at the community level. This highlights the model in which catecholamines act as selective ecological modulates, differentially influencing species based on their physiological abilities. Most previous work has focused on individual species or complex host-derived microbiomes, which makes it difficult to distinguish direct bacterial

responses to catecholamines from host-mediated effects. Therefore, by using a synthetic community, the present study shows that catecholamines can reshape microbial community composition even in the absence of host factors.

The enrichment of *Bacteroides* is particularly interesting when considered alongside the monoculture experiments. It is also important to recognize that changes in relative abundance in a community do not necessarily reflect absolute growth. The observed enrichment of *Bacteroides* is relative to the other species and it should be interpreted as changes in community structure rather than direct measures of growth. Nonetheless, increases in Bacteroidota-related species have been reported before. In a rodent model, Kelly et al. (2021) observed increased abundance of *Bacteroides* during chronic stress. Although we did not model host stress, catecholamine supplementation may mimic aspects of the neuroendocrine environment associated with stress responses.

Another species that showed consistent responses in the communities was *Klebsiella*. *Klebsiella* consistently decreased in relative abundance across all catecholamine treatments despite showing positive growth responses in isolation, particularly in polymer-catecholamine conditions. This finding was unexpected as members of the *Enterobacteriaceae*, including *Klebsiella pneumoniae* (Shpakov, 2009), have shown enhanced growth following exposure to noradrenaline (O'Donnell et al., 2006) and dopamine (Belay & Sonnenfeld, 2002). These were consistent in the dose response assay, which also varied with hemin availability. Since the relative abundance reflects the growth of all community members simultaneously, a decrease in *Klebsiella* does not necessarily indicate growth inhibition, but rather a reduced competitive position within the community. Nevertheless, the discrepancy between monoculture and community responses highlights the importance of ecological context in determining bacterial growth.

Unlike *Bacteroides* and *Klebsiella*, *Ruminococcus* and *Bifidobacterium* showed variation within the communities depending on the catecholamine present. However, both genera showed strong positive enrichment in relative abundance in dopamine treated communities, while exhibiting less or negative growth in the dose-response assays, and

varying enrichments in the polymer-hormone assay. These responses suggest that low abundance species may be particularly sensitive to catecholamine-mediated environmental changes in a community. The dominant genera showed stable abundance patterns, while the less abundant species exhibited pronounced treatment-specific shifts. This observation aligns more broadly with ecological studies indicating that low abundance microorganisms can respond strongly to environmental perturbations and contribute to community dynamics and functions (Jousset et al., 2017).

Taken together, the synthetic community reinforces the central finding of this study that catecholamine responses are highly context dependent. Across the monoculture experiments, bacterial growth responses varied according to species identity, hormone concentration, hemin, and polymer availability. However, in a community, these responses did not translate into community composition. These findings suggest that catecholamine mediated effects cannot be solely explained by direct stimulation or inhibition of individual bacterial species. Rather, the effects of catecholamines emerge from interactions between bacterial physiology and community structure. Therefore, the response of a species does not depend on its intrinsic ability to respond to catecholamines but also on how other species in a community respond to the same signal.

The differing responses between the catecholamines themselves also indicate that bacterial responses cannot simply be grouped under a universal effect of catecholamines. Although these compounds belong to the same neurochemical family, they frequently showed distinct growth patterns across monocultures and communities. The underlying mechanisms of these findings remain unclear, yet our data show that individual catecholamines have unique effects and bacteria also respond to specific neurochemicals differently. Many studies discuss bacterial responses to stress hormones collectively, yet this study shows that the effects of catecholamines or stress hormones may depend strongly on which catecholamine is present.

Furthermore, the differences in monoculture and community responses also highlight an important consideration when studying microbial endocrinology exclusively in isolated

cultures. While monocultures provide valuable mechanistic insight, they do not capture the complexity of community interactions where microbes respond simultaneously to an environment signal. The findings of this study show the community level outcomes cannot always be predicted from individual growth responses alone. Overall, this study demonstrates that host-associated catecholamines have the capacity to influence both individual bacterial species and microbial community composition in a highly species-specific and context-dependent manner.

Chapter 6: Limitations and Future Directions

The findings presented in this study provide new insights into the effects of catecholamines on gut bacterial growth and community composition, highlighting the complex and context-dependent nature of host–microbe neuroendocrine interactions. However, as with any experimental study, several limitations should be considered when interpreting the results.

A primary limitation of this study was that all experiments were conducted under *in vitro* conditions. While it is effective in understanding direct bacterial responses to catecholamines, they do not fully encapsulate the complexity of the gastrointestinal environment. In the human gut, bacterial populations are exposed to numerous host-derived factors including immune responses, mucus-associated nutrients, intestinal epithelial signaling (Thursby and Juge, 2017) which all contribute to fluctuating levels of neuroendocrine molecules. Therefore, the responses observed in this study may not fully reflect bacterial behavior within the human gut.

Furthermore, growth was used as a proxy for bacterial responses. Although growth provides valuable insight into overall effects of catecholamines, it does not reveal the underlying mechanism for the observed responses. Catecholamines are known to influence a variety of bacterial processes such as biofilm formation, gene expression, and motility (Sandrini et al., 2014; Oleskin et al. 2021). Therefore, changes in growth alone cannot determine whether it was driven by signaling pathways, metabolic regulation, or other mechanisms. Similarly, growth on polymers were used to infer polymer utilization potential. However, polymer degradation, enzyme activity and substrate consumption were not measured directly.

Although the study identified variation in bacterial responses across species and contexts, the molecular mechanisms still remain unresolved. The observations on varying catecholamine growth responses, hemin availability and CAZyme repertoires suggest that signaling and regulatory pathways may play a key role, however no transcriptomic, proteomic or metabolomic analyses were conducted to investigate these. As a result,

conclusions regarding catecholamine signaling, quorum sensing interactions and metabolic regulation remain speculative and require experimental validation.

The synthetic community provides a useful model in bridging monoculture and community responses. Nevertheless, it remains a simplified approximation of the intestinal microbiota. The community consisted of a limited number of species and lacked diversity and complexity *in vivo*. Furthermore, community composition was assessed using 16S rRNA gene amplicon sequencing which provides relative rather than absolute abundance data. This makes it difficult to distinguish if the observed shifts were changes in bacterial growth. Also, the taxonomic resolution of 16S sequencing is often not sufficient to differentiate closely related species and does not provide information on gene expression or functional responses to catecholamines.

Future directions should focus on identifying the molecular mechanisms underlying the species-specific responses observed in this study. Transcriptomic analyses such as RNA sequencing would help determine how catecholamines influence gene expression and also pathways involved in nutrient acquisition, stress responses, signaling and metabolism. Particular focus should be on the species that display contrasting behaviors across contexts such as *Bacteroides*, *Escherichia*, *Enterococcus* and *Ruminococcus*. Moreover, future studies should investigate the potential interactions between catecholamine signaling and bacterial communication systems such as quorum sensing. Experiments examining quorum sensing molecule production and signaling activity following catecholamine exposure may provide valuable insight into the regulatory networks of these species. Additionally, the effects of catecholamines on bacterial functions remain unclear. Future work should incorporate metabolomic approaches to understanding if community changes are accompanied by metabolite production, particularly short-chain fatty acids such as acetate, propionate, and butyrate, as these metabolites play important roles in host health.

Disclaimer

During the writing of this thesis, OpenAI ChatGPT-5.5 was used occasionally with text refinement and R programming for data analysis. The tool was used solely as a support and not as a substitute for independent scientific work. All generated content was reviewed and edited, as necessary. Therefore, I take full responsibility for the final content of this thesis.

References

Afzaal, Muhammad, Farhan Saeed, Yasir Abbas Shah, Muzzamal Hussain, Roshina Rabail, Claudia Terezia Socol, Abdo Hassoun et al. "Human gut microbiota in health and disease: Unveiling the relationship." *Frontiers in microbiology* 13 (2022): 999001.

Albani, G., Chellamuthu, V.R., Morlacchi, L., Zirone, F., Youssefi, M., Giardini, M., Chao, Y.X., Tan, E.K. and Albani, S., 2025. Gut Microbiota and Dopamine: Producers, Consumers, Enzymatic Mechanisms, and In Vivo Insights. *Bioengineering*, *13*(1), p.55.

Alou, M. T., Lagier, J. C., & Raoult, D. (2016). Diet influence on the gut microbiota and dysbiosis related to nutritional disorders. Human Microbiome Journal, 1, 3-11.

Bailey, M. T., Dowd, S. E., Parry, N. M., Galley, J. D., Schauer, D. B., & Lyte, M. (2010). Stressor exposure disrupts commensal microbial populations in the intestines and leads to increased colonization by Citrobacter rodentium. *Infection and immunity*, *78*(4), 1509-1519.

Basnet, J., Eissa, M. A., Yanes Cardozo, L. L., Romero, D. G., & Rezq, S. (2024). Impact of and prebiotics on gut microbiome and hormonal regulation. Gastrointestinal Disorders, 6(4), 801-815.

Boujnane, M., Boukerb, A. M., & Connil, N. (2024). Bacterial gene expression in response to catecholamine stress hormones. *Current Opinion in Endocrine and Metabolic Research*, *36*, 100543.

Boukerb, A. M., Cambronel, M., Rodrigues, S., Mesguida, O., Knowlton, R., Feuilloley, M. G., Zommiti & Connil, N. (2021). Inter-kingdom signaling of stress hormones: sensing, transport and modulation of bacterial physiology. *Frontiers in microbiology*, *12*, 690942.

Buffie, C. G., & Pamer, E. G. (2013). Microbiota-mediated colonization resistance against intestinal pathogens. *Nature Reviews Immunology*, *13*(11), 790-801.

Celis, A. I., Relman, D. A., & Huang, K. C. (2023). The impact of iron and heme availability on the healthy human gut microbiome in vivo and in vitro. *Cell chemical biology*, *30*(1), 110-126.

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: a bacterial adrenergic receptor. *Proceedings of the National Academy of Sciences*, *103*(27), 10420-10425.

Coyte, K. Z., & Rakoff-Nahoum, S. (2019). Understanding competition and cooperation within the mammalian gut microbiome. *Current Biology*, *29*(11), R538-R544.

Coyte, K. Z., Schluter, J., & Foster, K. R. (2015). The ecology of the microbiome: networks, competition, and stability. *Science*, *350*(6261), 663-666.

DeVries, J. W. (2004). Dietary fiber: the influence of definition on analysis and regulation. Journal of AOAC international, 87(3), 682-706.

Do Carmo, M. M., Walker, J. C., Novello, D., Caselato, V. M., Sgarbieri, V. C., Ouwehand, A. C., Andreollo, N. A., Hiane, P. A., & Dos Santos, E. F. (2016). Polydextrose: Physiological Function, and Effects on Health. *Nutrients*, *8*(9), 553.

Donaldson, G. P., Lee, S. M., & Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nature Reviews Microbiology*, *14*(1), 20-32.

Dowd, S. E. (2007). Escherichia coli O157: H7 gene expression in the presence of catecholamine norepinephrine. *FEMS microbiology letters*, *273*(2), 214-223.

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. Gut microbes, 3(4), 289-306.

Frawley, E. R., & Fang, F. C. (2014). The ins and outs of bacterial iron metabolism. *Molecular microbiology*, *93*(4), 609–616.

Freestone, P. P., Lyte, M., Neal, C. P., Maggs, A. F., Haigh, R. D., & Williams, P. H. (2000). The mammalian neuroendocrine hormone norepinephrine supplies iron for bacterial growth in the presence of transferrin or lactoferrin. *Journal of bacteriology*, *182*(21), 6091-6098.

Fu, J., Zheng, Y., Gao, Y., & Xu, W. (2022). Dietary fiber intake and gut microbiota in human health. Microorganisms, 10(12), 2507.

Fukui, H., Xu, X., & Miwa, H. (2018). Role of gut microbiota-gut hormone axis in the pathophysiology of functional gastrointestinal neurogastroenterology and motility, 24(3), 367.

Gill, S. K., Rossi, M., Bajka, B., & Whelan, K. (2021). Dietary fibre in gastrointestinal health and disease. Nature Reviews Gastroenterology & Hepatology, 18(2), 101-116.

Hamaker, B. R., & Tuncil, Y. E. (2014). A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. *Journal of molecular biology*, *426*(23), 3838-3850.

Hammouda, Z. K., Wasfi, R., & Abdeltawab, N. F. (2023). Hormonal drugs: Influence on growth, biofilm formation, and adherence of selected gut microbiota. *Frontiers in Cellular and Infection Microbiology*, *13*, 1147585.

Jones, J. M. (2014). CODEX-aligned dietary fiber definitions help to bridge the 'fiber gap'. Nutrition journal, 13(1), 34.

Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M.C., Rivett, D.W., Salles, J.F. and Van Der Heijden, M.G., 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. *The ISME journal*, *11*(4), pp.853-862.

Karavolos, M. H., & Khan, C. A. (2013). Host Neuroendocrine Stress Hormones Driving Bacterial Behaviour and Virulence. In *Moonlighting Cell Stress Proteins in Microbial Infections* (pp. 387-398).

Karl, J. P., Hatch, A. M., Arcidiacono, S. M., Pearce, S. C., Pantoja-Feliciano, I. G., Doherty, L. A., & Soares, J. W. (2018). Effects of psychological, environmental and physical stressors on the gut microbiota. *Frontiers in microbiology*, *9*, 2013.

Kelly, L.S., Apple, C.G., Gharaibeh, R., Pons, E.E., Thompson, C.W., Kannan, K.B., Darden, D.B., Efron, P.A., Thomas, R.M. and Mohr, A.M., 2021. Stress-related changes in the gut microbiome after trauma. *Journal of Trauma and Acute Care Surgery*, *91*(1), pp.192-199.

Kurokawa, K., Itoh, T., Kuwahara, T., Oshima, K., Toh, H., Toyoda, A., Takami, H., Morita, H., Sharma, V.K., Srivastava, T.P. and Taylor, T.D., 2007. Comparative metagenomics

revealed commonly enriched gene sets in human gut microbiomes. *Dna Research*, *14*(4), pp.169-181.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature*, *489*(7415), 220-230.

Lyte, M. (2004). Microbial endocrinology and infectious disease in the 21st century. Trends in microbiology, 12(1), 14-20.

Lyte, M. (2014). Microbial endocrinology: Host-microbiota neuroendocrine interactions influencing brain and behavior. *Gut microbes*, *5*(3), 381-389.

Lyte, M., & Ernst, S. (1992). Catecholamine induced growth of gram negative bacteria. *Life sciences*, *50*(3), 203-212.

Lyte, M., & Ernst, S. (1993). Alpha and beta adrenergic receptor involvement in catecholamine-induced growth of gram-negative bacteria. *Biochemical and biophysical research communications*, *190*(2), 447-452.

Makki, K., Deehan, E. C., Walter, J., & Bäckhed, F. (2018). The impact of dietary fiber on gut microbiota in host health and disease. Cell host & microbe, 23(6), 705-715.

Martens, E. C., Kelly, A. G., Tauzin, A. S., & Brumer, H. (2014). The devil lies in the details: how variations in polysaccharide fine-structure impact the physiology and evolution of gut microbes. *Journal of molecular biology*, *426*(23), 3851-3865.

Midani, F. S., Collins, J., & Britton, R. A. (2021). AMiGA: software for automated analysis of microbial growth assays. *Msystems*, *6*(4), 10-1128.

Miethke, M., & Marahiel, M. A. (2007). Siderophore-based iron acquisition and pathogen control. *Microbiology and molecular biology reviews*, *71*(3), 413-451.

Neuman, H., Debelius, J. W., Knight, R., & Koren, O. (2015). Microbial endocrinology: the interplay between the microbiota and the endocrine system. FEMS microbiology reviews, 39(4), 509-521.

Niu, L., Gao, M., Wen, S., Wang, F., Shangguan, H., Guo, Z., Zhang, R., & Ge, J. (2023). Effects of Catecholamine Stress Hormones Norepinephrine and Epinephrine on Growth, Antimicrobial Susceptibility, Biofilm Formation, and Gene Expressions of

Enterotoxigenic Escherichia coli. International Journal of Molecular Sciences, 24(21), 15646.

Nixon, B. T., Ronson, C. W., & Ausubel, F. M. (1986). Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes ntrB and ntrC. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(20), 7850–7854.

O'Donnell, P.M.; Aviles, H.; Lyte, M.; Sonnenfeld, G. Enhancement of In Vitro Growth of Pathogenic Bacteria by Norepinephrine: Importance of Inoculum Density and Role of Transferrin. Appl. Environ. Microbiol. 2006, 72, 5097–5099.

Oleskin, A. V., Sorokina, E. V., & Shilovsky, G. A. (2021). Interaction of catecholamines with microorganisms, neurons, and immune cells. *Biology Bulletin Reviews*, *11*(4), 358-367.

Oleskin, A. V., Sorokina, E. V., & Zarubina, A. P. (2017). Testing neurotransmitters for toxicity with a luminescent biosensor: implications for microbial endocrinology. *Journal of Pharmacy and Nutrition Sciences*, *7*, 88-94.

Perraud, Q., Kuhn, L., Fritsch, S., Graulier, G., Gasser, V., Normant, V., ... & Schalk, I. J. (2022). Opportunistic use of catecholamine neurotransmitters as siderophores to access iron by Pseudomonas aeruginosa. *Environmental microbiology*, *24*(2), 878-893.

Peter, J., Fournier, C., Durdevic, M., Knoblich, L., Keip, B., Dejaco, C., Trauner, M. and Moser, G., 2018. A microbial signature of psychological distress in irritable bowel syndrome. *Biopsychosocial Science and Medicine*, *80*(8), pp.698-709.

Prah, A., & Mavri, J. (2024). L-DOPA Autoxidation: An Empirical Valence Bond Simulation of the Reactive Step. *The Journal of Physical Chemistry B*, *128*(35), 8355-8361.

Rakoff-Nahoum, S., Coyne, M. J., & Comstock, L. E. (2014). An ecological network of polysaccharide utilization among human intestinal symbionts. *Current Biology*, *24*(1), 40-49.

Safarchi, A., Al-Qadami, G., Tran, C. D., & Conlon, M. (2025). Understanding dysbiosis and resilience in the human gut microbiome: Biomarkers, interventions, and challenges. *Frontiers in Microbiology*, *16*, 1559521.

Sandrini, S. M., Shergill, R., Woodward, J., Muralikuttan, R., Haigh, R. D., Lyte, M., & Freestone, P. P. (2010). Elucidation of the mechanism by which catecholamine stress hormones liberate iron from the innate immune defense proteins transferrin and lactoferrin. *Journal of bacteriology*, *192*(2), 587-594.

Sandrini, S., Alghofaili, F., Freestone, P., & Yesilkaya, H. (2014). Host stress hormone norepinephrine stimulates pneumococcal growth, biofilm formation and virulence gene expression. *BMC microbiology*, *14*(1), 180.

Schaible, U. E., & Kaufmann, S. H. (2004). Iron and microbial infection. *Nature Reviews Microbiology*, *2*(12), 946-953.

Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J., & Duncan, S. H. (2013). The influence of diet on the gut microbiota. Pharmacological research, 69(1), 52-60.

Shen, S., Prame Kumar, K., Wen, S. W., Shim, R., Wanrooy, B. J., Stanley, D., ... & Wong, C. H. (2021). Deficiency of dietary fiber modulates gut microbiota composition, neutrophil recruitment and worsens experimental colitis. Frontiers in Immunology, 12, 619366.

Shpakov, A. O. (2009). QS-type bacterial signal molecules of nonpeptide origin. *Microbiology*, *78*(2), 133-143.

Sommer, F., & Bäckhed, F. (2013). The gut microbiota—masters of host development and physiology. *Nature reviews microbiology*, *11*(4), 227-238.

Sperandio, V., Torres, A. G., & Kaper, J. B. (2002). Quorum sensing Escherichia coli regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in E. coli. *Molecular microbiology*, *43*(3), 809-821.

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical journal*, *474*(11), 1823-1836.

Tomasello, G., Mazzola, M., Bosco, V., Tomasello, G., Damiani, P., Sinagra, E., & Carini, F. (2018). Intestinal dysbiosis and hormonal neuroendocrine secretion in the

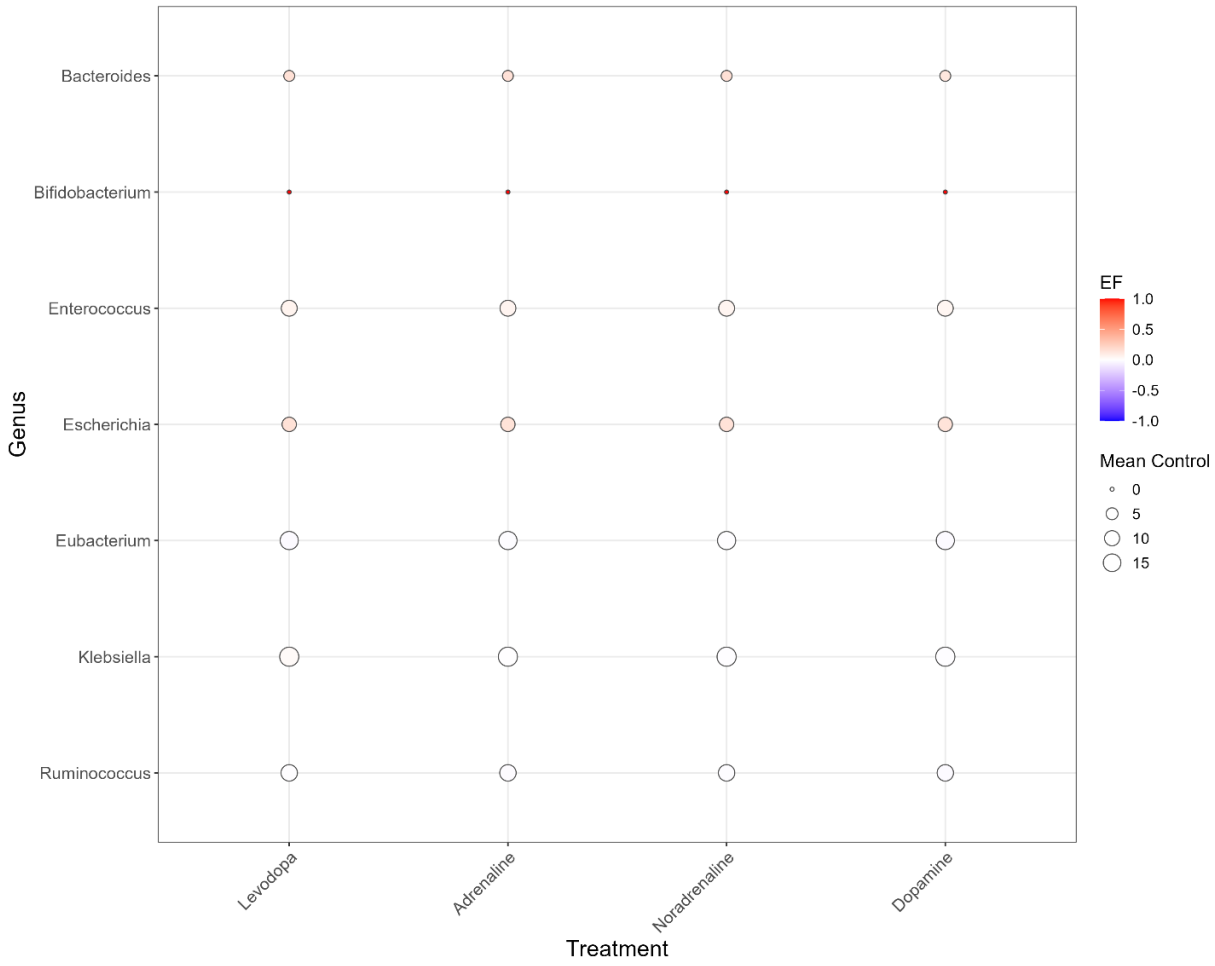
fibromyalgic patient. Biomedical Papers of the Medical Faculty of Palacky University in Olomouc, 162(4).

Wardman, J. F., Bains, R. K., Rahfeld, P., & Withers, S. G. (2022). Carbohydrate-active enzymes (CAZymes) in the gut microbiome. *Nature Reviews Microbiology*, *20*(9), 542-556.

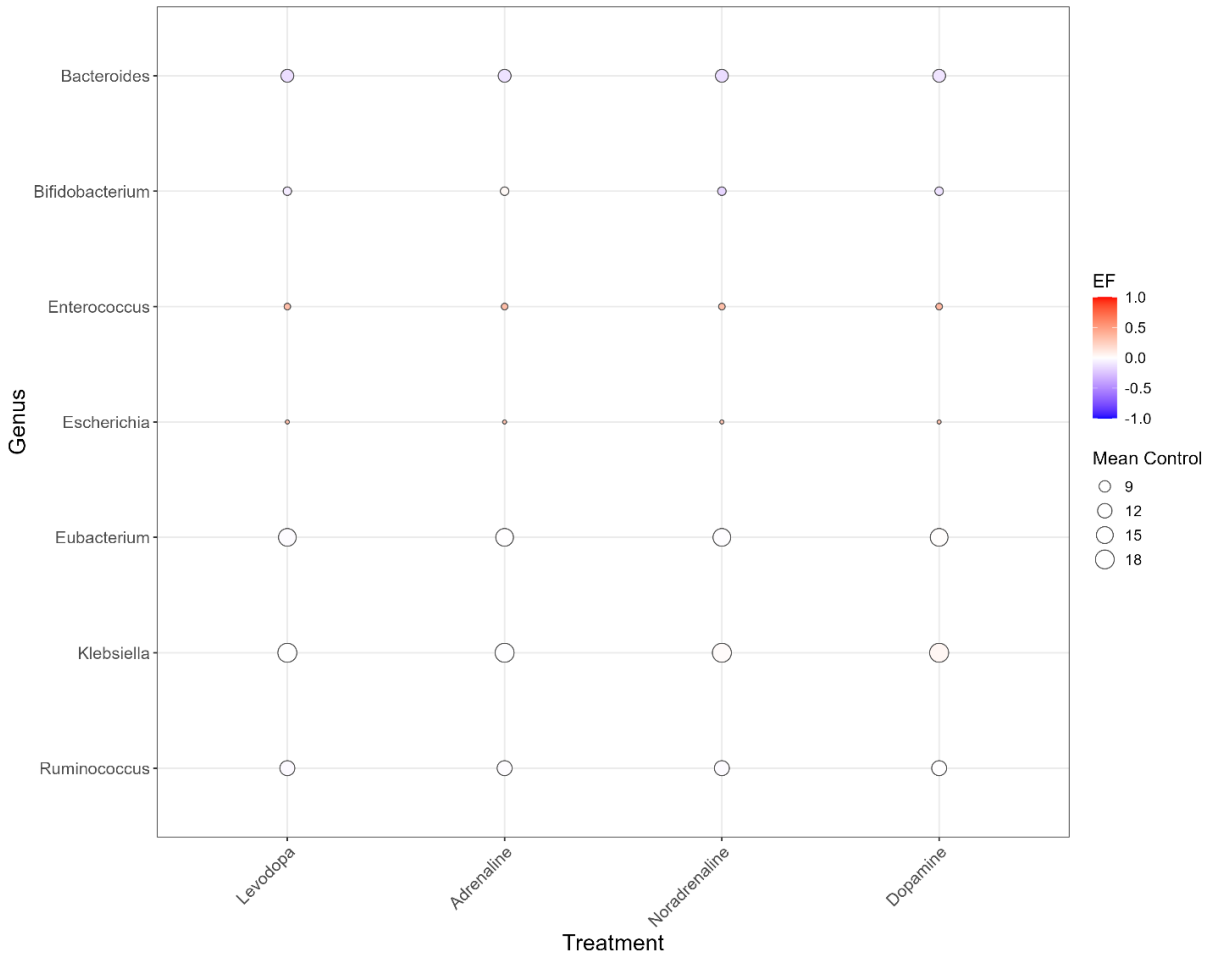
Yin, Y., Mao, X., Yang, J., Chen, X., Mao, F., & Xu, Y. (2012). dbCAN: a web resource for automated carbohydrate-active enzyme annotation. *Nucleic acids research*, *40*(W1), W445-W451.

Ze, X., Duncan, S. H., Louis, P., & Flint, H. J. (2012). Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. *The ISME journal*, *6*(8), 1535-1543.

Supplementary Materials

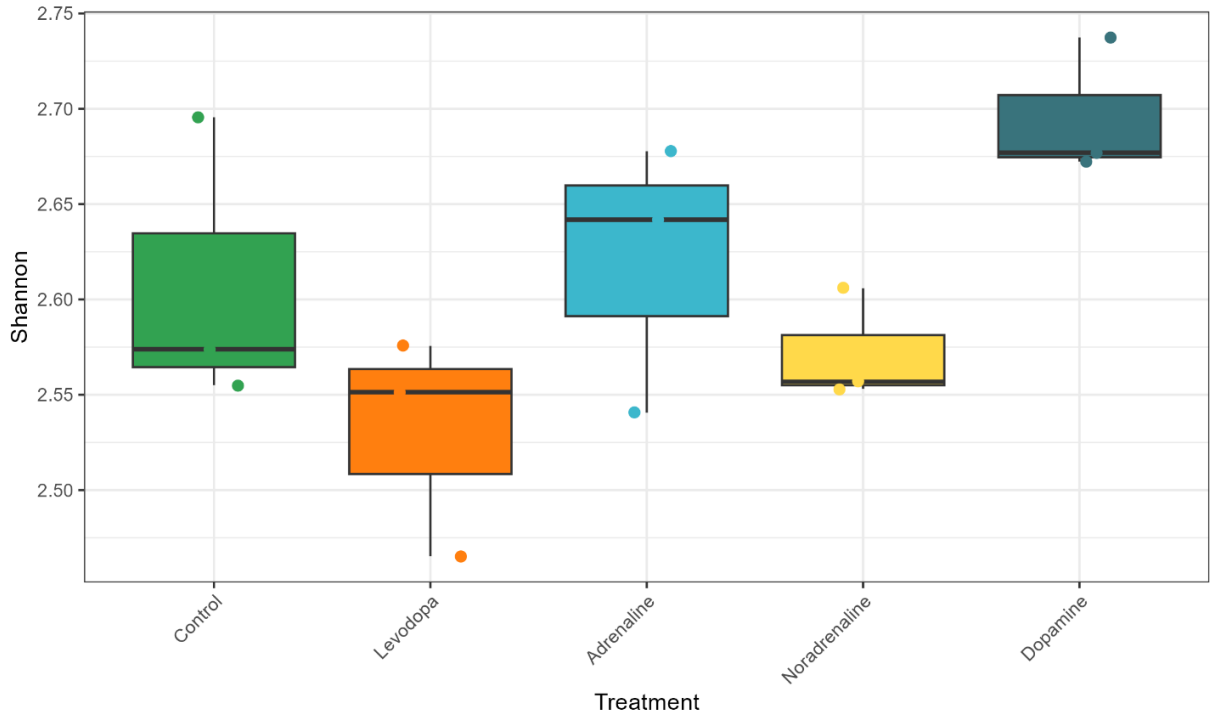


Supplementary Figure S1: Genus level enrichments in species across catecholamines without hemin *Bubble plot representing changes in genus-level across pooled concentrations of catecholamines. The color represents the enrichment factor (EF) where red = enrichment and blue = depletion. The size of the bubble reflects the relative abundance in control samples where larger bubble size = more abundant in control and smaller bubble = less abundant in control.*

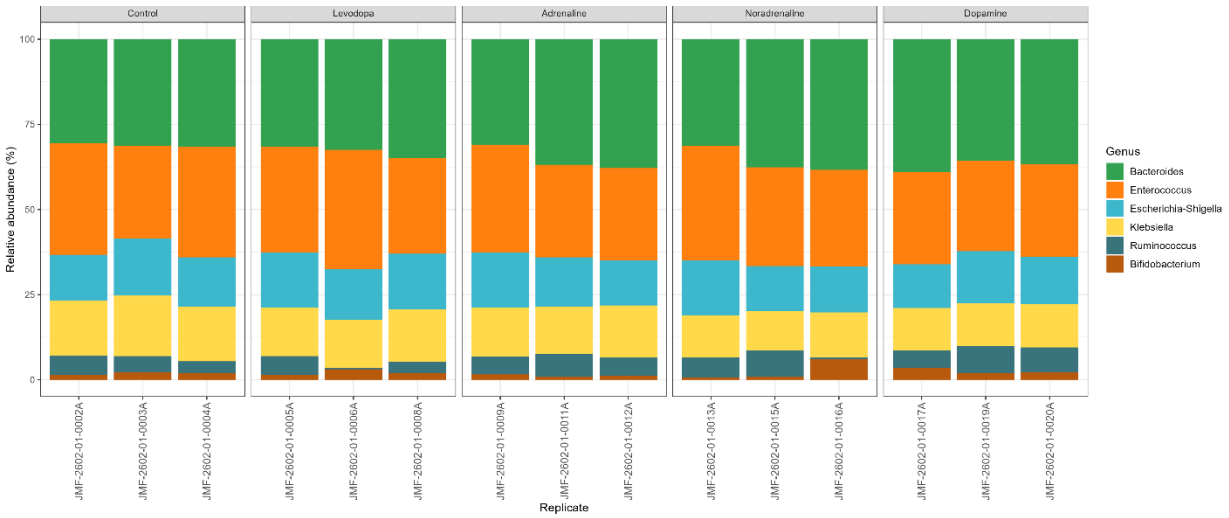


Supplementary Figure S2: Genus level enrichments in species across catecholamines with hemin supplementation

Bubble plot representing changes in genus-level across pooled concentrations of catecholamines. The color represents the enrichment factor (EF) where red = enrichment and blue = depletion. The size of the bubble reflects the relative abundance in control samples where larger bubble size = more abundant in control and smaller bubble = less abundant in control.

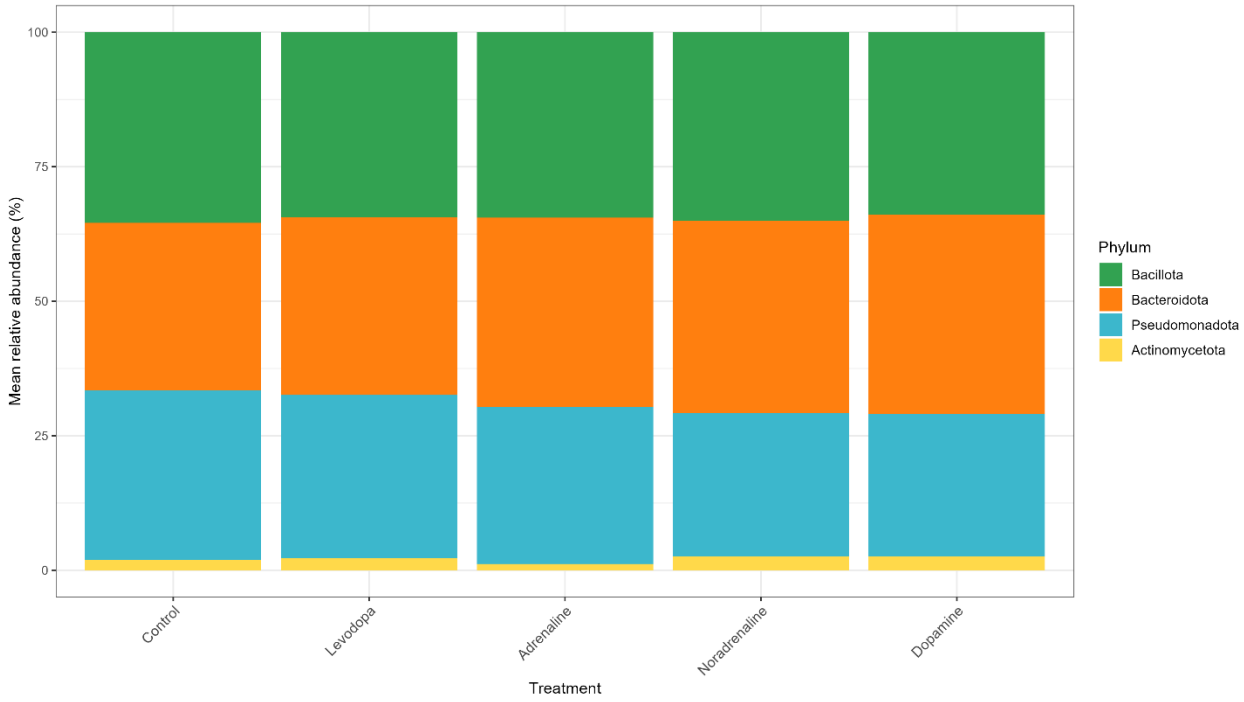


Supplementary Figure S3: Alpha diversity of the synthetic microbial community
Boxplot showing the distribution of Shannon diversity indices for the 12-species synthetic microbial community (SynCom) across the Control, Levodopa, Adrenaline, Noradrenaline, and Dopamine treatment groups. The x-axis represents the treatment groups, while the y-axis shows the Shannon diversity index, a measure of community alpha diversity that incorporates both species richness and evenness. Each box represents the interquartile range (IQR), the horizontal line within each box indicates the median value, whiskers represent spread of the data, and individual points correspond to biological replicates. Higher Shannon index values indicate greater community diversity

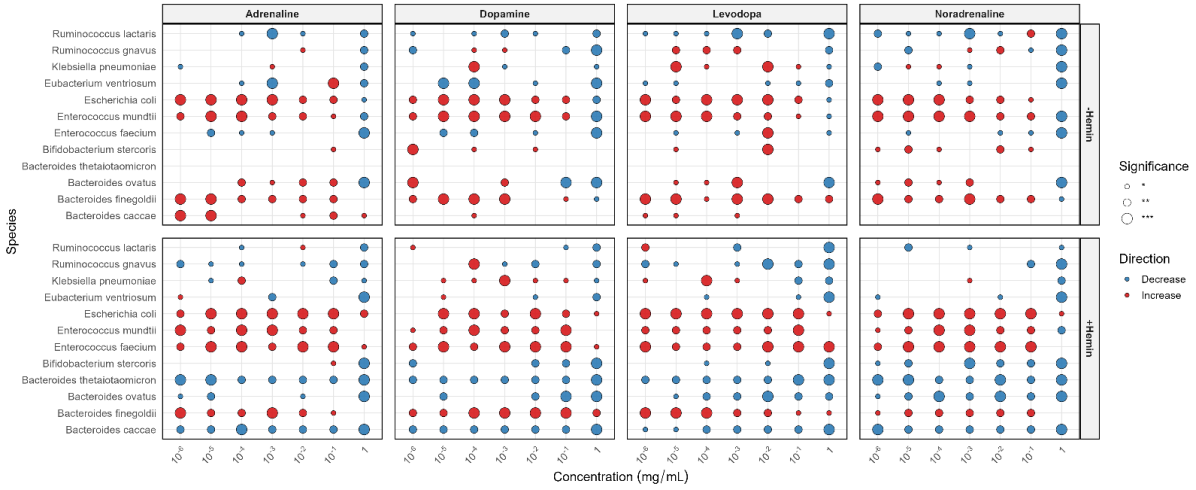


Supplementary Figure S4: Relative abundance of bacterial species across individual replicates and treatment groups

Faceted stacked bar plots showing the relative abundance (%) of bacterial species within individual biological replicates of the synthetic communities across the treatment groups. Each panel represents a treatment condition, and each stacked bar corresponds to a single replicate sample. The x-axis represents the sample IDs of the individual replicates, while the y-axis indicates relative abundance (%). Different colors represent the detected bacterial genera as shown in the legend. Each bar is scaled to 100%, illustrating the proportional contribution of each genus to the overall community composition within each replicate.

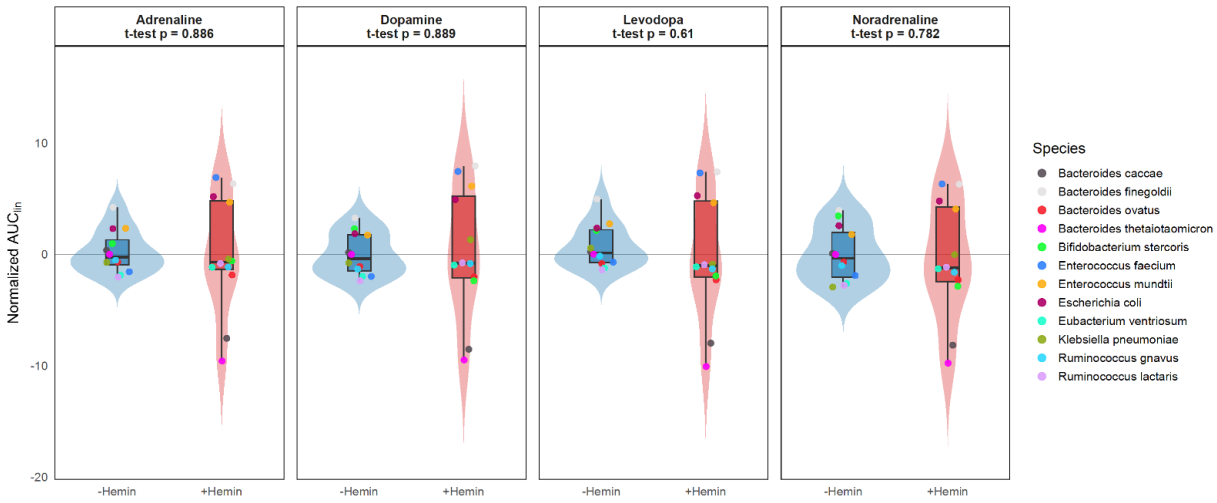


Supplementary Figure S5: Phylum-level distribution of the synthetic microbial community
Stacked bar plot showing the mean relative abundance (%) of bacterial phyla within the synthetic communities across the treatment groups. The x-axis represents the treatment groups, while the y-axis indicates mean relative abundance (%). Different colors represent the phyla as shown in the legend. Each bar is scaled to 100%, illustrating the proportional contribution of each phylum to the overall community composition within each treatment group.



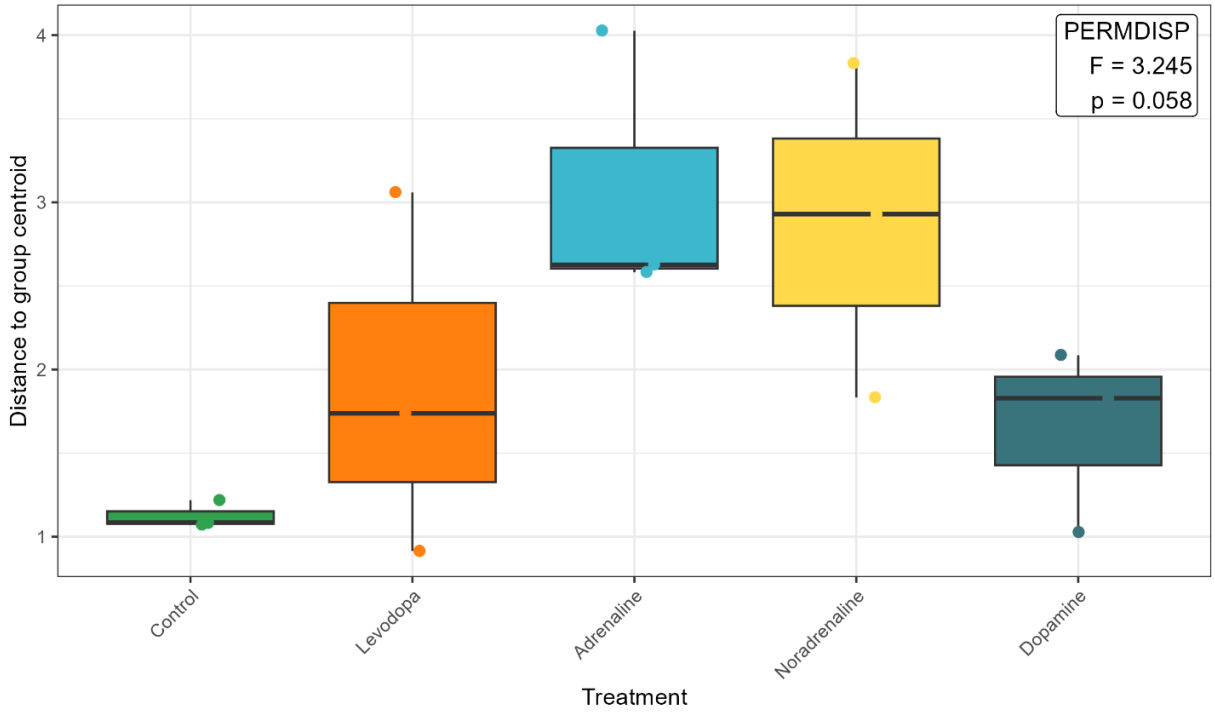
Supplementary Figure S6: Significant dose- and hemin- dependent modulation of bacterial growth

The dot plot shows the significant growth responses of the 12 bacterial species in increasing concentrations of adrenaline, dopamine, levodopa and noradrenaline. Data is further faceted by the supplementation of hemin. The color of the dots indicates the direction of the response, with red representing a significant increase and blue representing a significant decrease. The size of the dot corresponds to the degree of statistical significance resulted through a Welch's t-test where a larger dot indicates a higher level of significance.



Supplementary Figure S7: Overall impact of hemin on catecholamine-induced growth

The violin plot shows the distribution of normalized area under the curve (AUC) for the 12 bacterial species where the data of the individual concentrations (10^{-6} to 1 mg/mL) have been collapsed for each compound. The plot is an illustration of contrast growth dynamics with the supplementation of hemin. The individual data points for each species is the mean response across the concentrations. The box plots show the median and interquartile region (IQR) with whiskers extending representing the spread of the data. The shading around the box plot shows the density of the data points at that specific value where a 'wider' shading indicates many species having similar growth and a 'narrower' shading indicating fewer species with similar responses.



Supplementary Figure S8: PERMDISP analysis of synthetic community dispersion across treatment groups. *Boxplot showing the distances of individual samples to their respective treatment group centroids based on Aitchison distance. The x-axis represents the treatment groups, while the y-axis shows the distance of each sample to its group centroid, where larger values indicate greater variation within the group in community composition. Boxes represent the interquartile range (IQR), horizontal lines indicate the median, whiskers show the spread of the data, and points represent individual biological replicates. Multivariate homogeneity of group dispersions was assessed using PERMDISP, which indicated no significant differences in dispersion among treatment groups (F = 3.245, p = 0.058)*

Genus	Hormone	EF	P value	FDR
Bifidobacterium	Levodopa	1	0.049544	0.115601797
Bacteroides	Levodopa	0.156485	0.263221	0.368509002
Escherichia	Levodopa	0.15414	0.007737	0.043325435
Enterococcus	Levodopa	0.059996	0.035489	0.110228274
Klebsiella	Levodopa	0.026974	0.010382	0.04844838
Ruminococcus	Levodopa	-0.01027	0.398284	0.484867372
Eubacterium	Levodopa	-0.0199	0.018185	0.063645905
Bifidobacterium	Adrenaline	1	0.049544	0.115601797
Bacteroides	Adrenaline	0.156036	0.246286	0.368509002
Escherichia	Adrenaline	0.14853	0.007737	0.043325435
Enterococcus	Adrenaline	0.058764	0.054478	0.117336834
Klebsiella	Adrenaline	-0.00363	1	1
Eubacterium	Adrenaline	-0.01415	0.078609	0.146736308
Ruminococcus	Adrenaline	-0.01708	0.602233	0.624538143
Bifidobacterium	Noradrenaline	1	0.007657	0.043325435
Bacteroides	Noradrenaline	0.161083	0.257735	0.368509002
Escherichia	Noradrenaline	0.150978	0.007737	0.043325435
Enterococcus	Noradrenaline	0.052499	0.069495	0.138990963
Klebsiella	Noradrenaline	-0.00863	0.392949	0.484867372
Eubacterium	Noradrenaline	-0.00971	0.513579	0.575208791
Ruminococcus	Noradrenaline	-0.01363	0.602233	0.624538143
Bifidobacterium	Dopamine	1	0.017439	0.063645905
Escherichia	Dopamine	0.144499	0.007737	0.043325435
Bacteroides	Dopamine	0.128279	0.295324	0.393765623
Enterococcus	Dopamine	0.048817	0.094641	0.16562196
Klebsiella	Dopamine	-0.00974	0.247766	0.368509002
Eubacterium	Dopamine	-0.02161	0.039367	0.110228274
Ruminococcus	Dopamine	-0.02233	0.461209	0.538077334
Enterococcus	Levodopa (H)	0.338306	0.000112	0.000780792
Escherichia	Levodopa (H)	0.333558	0.007737	0.021662717

Klebsiella	Levodopa (H)	0.000539	0.392949	0.484867372
Eubacterium	Levodopa (H)	-0.01592	0.078609	0.137565288
Ruminococcus	Levodopa (H)	-0.02379	0.145464	0.226277074
Bifidobacterium	Levodopa (H)	-0.08411	0.513579	0.599175823
Bacteroides	Levodopa (H)	-0.14141	0.064732	0.129463167
Enterococcus	Adrenaline (H)	0.340339	0.000112	0.000780792
Escherichia	Adrenaline (H)	0.313619	0.007737	0.021662717
Bifidobacterium	Adrenaline (H)	0.032925	0.392949	0.484867372
Eubacterium	Adrenaline (H)	-0.00236	0.58042	0.624538143
Klebsiella	Adrenaline (H)	-0.00597	0.58042	0.624538143
Ruminococcus	Adrenaline (H)	-0.0109	0.602233	0.624538143
Bacteroides	Adrenaline (H)	-0.12375	0.026568	0.061991973
Enterococcus	Noradrenaline (H)	0.335902	0.000112	0.000780792
Escherichia	Noradrenaline (H)	0.30598	0.007737	0.021662717
Klebsiella	Noradrenaline (H)	0.018747	0.078609	0.137565288
Eubacterium	Noradrenaline (H)	-0.00908	0.291293	0.42927367
Ruminococcus	Noradrenaline (H)	-0.01558	0.398284	0.484867372
Bacteroides	Noradrenaline (H)	-0.14375	0.015421	0.039254201
Bifidobacterium	Noradrenaline (H)	-0.17828	0.007737	0.021662717
Enterococcus	Dopamine (H)	0.374412	0.000112	0.000780792
Escherichia	Dopamine (H)	0.319593	0.007737	0.021662717
Klebsiella	Dopamine (H)	0.047608	0.007737	0.021662717
Eubacterium	Dopamine (H)	0.011837	0.392949	0.484867372
Ruminococcus	Dopamine (H)	-0.00287	0.679344	0.679343635
Bacteroides	Dopamine (H)	-0.11377	0.129834	0.213844637
Bifidobacterium	Dopamine (H)	-0.12483	0.039367	0.08479098

Supplementary Table S1: Enrichment statistics table of dose-response assay

Genus level enrichments for pooled concentrations in dose response assay with the relevant hormones treatment (with Hemin are indicated as (H)) are shown, together with enrichment factor (EF), P values, and false discovery rate (FDR)-adjusted P values. Positive EF values indicate enrichment under treatment relative to control, whereas negative EF values indicate depletion.

Genus	Treatment	EF	P value	FDR
Bifidobacterium	Levodopa	0.0781143	1	1
Bacteroides	Levodopa	0.02904805	0.0808556	0.2425668
Escherichia	Levodopa	0.02715986	0.66252058	0.99378088
Enterococcus	Levodopa	0.00896779	1	1
Klebsiella	Levodopa	-0.06391317	0.0808556	0.2425668
Ruminococcus	Levodopa	-0.20617577	0.38273309	0.76546618
Ruminococcus	Adrenaline	0.11483515	0.38273309	0.45927971
Bacteroides	Adrenaline	0.06146679	0.38273309	0.45927971
Escherichia	Adrenaline	-0.00903842	0.66252058	0.66252058
Enterococcus	Adrenaline	-0.03657743	0.19043026	0.38086053
Klebsiella	Adrenaline	-0.06928677	0.0808556	0.38086053
Bifidobacterium	Adrenaline	-0.21991356	0.19043026	0.38086053
Bifidobacterium	Noradrenaline	0.14809808	0.66252058	0.7950247
Bacteroides	Noradrenaline	0.06905033	0.38273309	0.76546618
Ruminococcus	Noradrenaline	0.00748659	0.66252058	0.7950247
Enterococcus	Noradrenaline	-0.0076802	1	1
Escherichia	Noradrenaline	-0.0235145	0.38273309	0.76546618
Klebsiella	Noradrenaline	-0.14395545	0.0808556	0.48513359
Ruminococcus	Dopamine	0.19688068	0.19043026	0.2856454
Bifidobacterium	Dopamine	0.15039031	0.38273309	0.45927971
Bacteroides	Dopamine	0.08731945	0.0808556	0.2425668
Escherichia	Dopamine	-0.03332564	0.66252058	0.66252058
Enterococcus	Dopamine	-0.06568358	0.19043026	0.2856454
Klebsiella	Dopamine	-0.14133881	0.0808556	0.2425668

Supplementary Table S2: Enrichment statistics table of synthetic community
Enrichment factor (EF), P values, and false discovery rate (FDR)-adjusted P values for each genus under the relevant treatment conditions are shown. Positive EF values indicate enrichment under treatment relative to control, whereas negative EF values indicate depletion. No comparisons remained statistically significant after FDR correction.