Article

Gut Microbiota as a Source of Uremic Toxins

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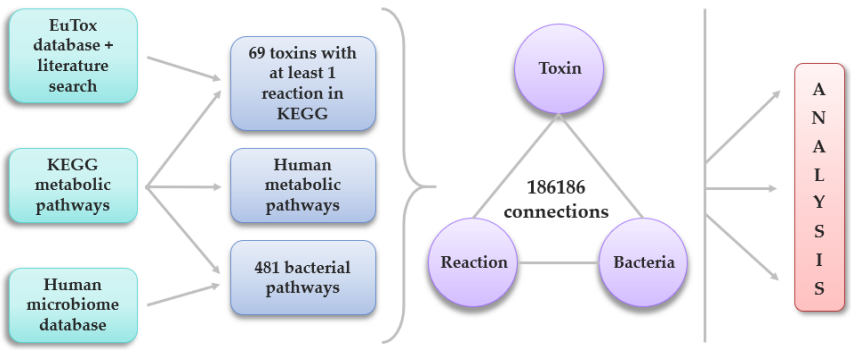
Abstract: Uremic toxins are the compounds that emerge in the blood when kidney excretory function is impaired. The cumulative detrimental effect of uremic toxins results in numerous health problems and eventually death during acute or chronic uremia, especially in end-stage renal disease. More than 100 different solutes rise during uremia; however, the exact origin for most of them is still discussable. There are 3 main sources for such compounds: exogenous ones are consumed with food, whereas endogenous are produced by host metabolism or by symbiotic microbiota metabolism. In this article, we identified uremic toxins presumably of gut microbiota origin. We analyzed various databases to get information on enzymatic reactions in bacteria and human organism potentially yielded uremic toxins and to determine what toxins could be synthetized in bacteria residing in human gut. We selected biochemical pathways resulting in uremic toxins synthesis related to specific bacterial strains, and revealed links between toxin concentration in uremia and the proportion of different bacteria species, which can synthesize it. Moreover, we defined the relative abundance of human toxin-generating enzymes as well as the possibility of a particular toxin synthesis by the human metabolism. Finally, we analyzed which bacteria are potentially producing the biggest number of uremic toxins as well as which bacteria are decomposing them. Our study presents a bioinformatics-based approach for both elucidation of the origin of uremic toxins and search of the most likely human microbiome producers of toxins that can be targeted and used for the therapy of adverse consequences of uremia.

Keywords: uremia, uremic toxins, microbiome, chronic kidney disease



1. Introduction

Chronic kidney disease (CKD) is a common health problem in adults defined as a gradual loss in kidney function. It affects about 10 % of the human population around the world [1]. Symptoms of CKD include reduced glomerular filtration rate, enhanced urinary

albumin excretion [2], and accumulation of many waste metabolic products in an organism that are normally excreted predominantly by the kidneys. These metabolites are called uremic toxins [3]. Their accumulation causes a great number of pathologies which are collectively named uremic syndrome or uremia [4]. The complications of uremia include multi-organ dysfunctions such as bone diseases, serositis, insulin resistance, renal fibrosis, podocyte dysfunction, decreased mental acuity, and a variety of cardiovascular problems [4,5]. Some of these ailments are present in the World Health Organization list of widespread death causes. Despite the severity of the uremia consequences and intense studying of the topic, the cellular and molecular mechanisms underlying syndrome development mostly remain unclear. The main reason for this is a huge and continuously expanding list of uremic toxins [6], which complicates the analysis of the impact of each of them and figuring possible interplays out. Nowadays, the European Uremic Solutes Database (EUTox-DB), which was created by the European Work Group on Uremic Toxins (EUTox), contains 130 solutes [7–10]. The substances belong to different chemical classes and participate in a great diversity of biochemical pathways, which makes further classification difficult.

Taking into account the fact that the human gut metagenome contains 150 times more genes than the host [11], it is not surprising that intestinal bacteria produce a huge variety of unique substances and those related to uremic toxins could be produced as well. Indeed, there are some observations that the gut microbiota contributes to uremic toxins production that inevitably aggravates the health status of CKD patients. Thus, as early as 1966, Einheber and Carter removed kidneys from germ-free rats and rats with normal microflora, thus creating rats that were not able to excrete uremic toxins and died because of uremia. Remarkably, germ-free animals stayed alive significantly longer than those with the normal gut microbiome, or than conventionalized germ-free [12]. Comparison of mural plasma from germ-free and conventional-microbiota rats revealed the emergence of many uremic compounds, including indole-3-propionic acid, indoxyl sulfate, and p-cresylsulfate in the conventional ones after nephrectomy which were less abundant in germ-free animals [13]. Similarly, the microbiota was found to be important in the health/disease balance under renal failure in humans as well. It was shown that hemodialysis patients who underwent a colon resection developed less severe uremia, whereas dialysis patients without such surgery accumulated more than 30 additional substances in plasma. Those substances were assigned as gut-derived uremic toxins [14].

Today the list of microbiota-derived toxins is growing. Bacterial origin of such substances as p-cresyl sulfate, indoxyl sulfate, indole-3-acetic acid, trimethylamine, trimethylamine-N-oxide, hippuric acid, phenol, phenylacetic acid have been proved in several independent experiments [15–18]. However, most compounds in EUTox-DB are under-explored. According to the Human Metabolome Database, 69 uremic toxins are classified as endogenous and 56 have not been classified yet [14,19–22].

In this study, we have analyzed Human Metabolome, Kyoto Encyclopedia of Genes and Genomes (KEGG), MetaCyc, and Human Protein Atlas databases to find microbial biochemical pathways and enzymes strongly associated with uremic toxin synthesis.

2. Results

For the analysis, we used a list of 142 substances attributed to uremic toxins. There were 130 solutes described in the Eutox database and additional 12 compounds referred to in publications [15,23,24] as potentially uremic toxins. Among them, 54 compounds were found in the KEGG database as products or participants of some biochemical reactions, and these uremic toxins were included in further analysis.

Using data from KEGG, we assigned each toxin to a specific enzymatic reaction in which it is a product or substrate. Then, using the NIH Human Microbiome Project database, all the bacteria in the human microbiome were identified. Lastly, all metabolic pathways for these bacteria were found in KEGG. As a result, we have obtained a

complete list of toxins with enzymatic pathways found in bacteria of the human microbiome, which can run these reactions.

Full data of bacterial synthetic and degrading enzymatic reactions for uremic toxins is available in Supplemental Table 1, with detailed data on bacterial strains, involved KEGG reactions, and included 186186 toxin-reaction-bacteria links. These data were then subjected to more in-depth analysis, the results of which are presented below.

Toxins, which can be synthesized by the microbiome

In Table 1, we have summarized the data on toxins associated with the human gut microbiome: the number of bacteria from the gut microbiome, which have reactions described in KEGG for synthesis or metabolism of given toxin, the number of different reactions for the given toxin in bacteria, the number of KEGG-described enzymatic reactions for the given toxin in the human organism.

Based on the KEGG database, we revealed that only 8 uremic toxins have no attributed pathways in human metabolism and vice versa have ascribed enzymes in bacteria (Table 1, and extended version in Supplemental Table 2). These compounds are mannitol, phenol, trimethylamine, oxalate, creatinine, trimethylamine-N-oxide, pseudouridine, 3-(3-hydroxyphenyl) propanoic acid. It should be noted that our analysis includes only described in KEGG enzymatic reactions, and thus compounds like creatinine or oxalate, which can be produced non-enzymatically in human organisms, fall in this category.

Table 1. Potential synthesis and metabolism of uremic toxins by microbiome bacteria species and human organism

Toxin	Number of synthesizing or metabolizing bacteria species	Number of different reactions in KEGG	Number of enzymes in human
Mannitol	56	4	**0**
Phenol	37	4	**0**
Trimethylamine	19	4	**0**
Oxalate	20	3	**0**
Creatinine	74	2	**0**
Trimethylamine-N-oxide	16	2	**0**
Pseudouridine	20	1	**0**
3-(3-Hydroxyphenyl) propanoic acid	13	1	**0**
S-Adenosylhomocysteine	143	35	1
Homocysteine	141	12	1
Argininic Acid	143	11	1
Putrescine	119	11	1
Methylglyoxal	138	10	1
Hypoxanthine	137	9	1
Urea	69	9	1
Xanthine	140	7	1
Nicotinamide	140	7	1
Cytidine	138	7	1
Uridine	138	7	1
anthranilic acid	108	7	1

Inosine	142	6	1
Indole-3-acetic acid (free)	117	6	1
3-hydroxyanthranilic acid	70	6	1
a-keto-d-Guanidinovaleric Acid	27	6	1
Myoinositol	116	5	1
Phenylacetic acid	106	5	1
Sorbitol	94	5	1
Dimethylamine	15	5	1
Orotic Acid	143	4	1
Xanthosine	142	4	1
y-guanidinobutyric Acid	59	4	1
Monomethylamine	22	4	1
p-Cresylsulfate (free)	81	3	1
Uric Acid	34	3	1
Kinurenine	20	3	1
Orotidine	143	2	1
Quinolinic Acid	89	2	1
Creatine	44	2	1
Gentisic acid	12	2	1
N-Acetylhistamine	82	1	1
Hippuric acid (total)	12	1	1
Taurocyamine	9	1	1
Melatonin	2	1	1
Ethylamine	1	1	1

We have also tested whether the number of potential toxin-synthesizing bacteria correlated with the toxin concentrations in either healthy or uremic conditions. However, the correlation coefficients for both conditions were non-significant and near-zero and thus no strong correlation was observed between toxin concentration and the number of bacteria producing it.

Toxins with the least abundant synthesizing human enzymes

Besides uremic toxins, which have no annotated synthesizing enzymes in *Homo sapiens*, we found 33 toxins for which few enzymes (from 1 to 3 enzymes) could be assigned as synthetic in the human organism (Table 1). To test whether these enzymes provide meaningful production of given toxins in humans we evaluated the amount of these enzymes in the human body.

From Human Protein Atlas database, we have extracted the data on mRNA abundance in different tissues and normalized it on average tissue weight. Using this approach, we obtained an approximate abundance of particular enzymes in the human organism. All human genes expression abundance in the whole organism demonstrates bimodal distribution with a large portion of genes being poorly represented and others of almost normal distribution. Green dashes indicate the position of uremic toxins-synthesizing enzymes (Fig. 1). According to our analysis, the most abundant enzymes are responsible for the synthesis of creatine, indole-3-acetic acid, nicotinamide, methylglyoxal, S-adenosylhomocysteine, and thus these toxins are expected to be predominantly produced

by the human organism. The five least abundant enzymes are responsible for the synthesis of melatonin, hexanal, orotidine, a-keto-d-guanidinovaleric acid, and 3-hydroxyanthranilic acid. Based on these results, we suggested that human metabolism might play an insignificant role in the production of these five compounds and they could be mostly produced by the microbiome.

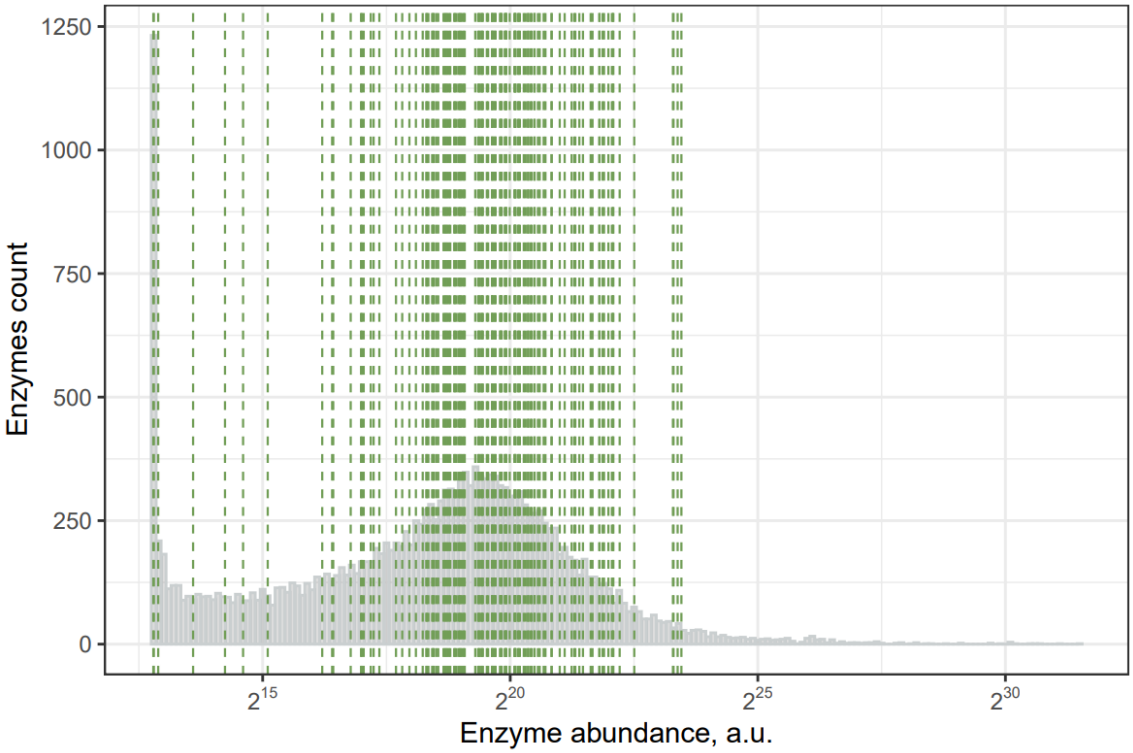


Figure 1: Abundance of human mRNA expression in the whole organism, green lines — genes of uremic toxin synthesizing enzymes

Bacteria with the ability to synthesize or metabolize uremic toxins

Data from KEGG also allowed us to address the questions on different bacteria's ability to synthesize uremic toxins. Using KEGG, it is possible to extract the data whether a toxin is "upstream" or "downstream" the certain pathway in a reaction, which theoretically should correlate with toxin being synthesized or metabolized in a given reaction. Using KEGG data, we have summed the number of toxins that certain bacteria can potentially synthesize or metabolize. Full data for 142 bacteria taxa is available in Supplemental Table 3. From this list, 70 bacteria are potentially capable of synthesizing more than 20 uremic toxins, while only 20 species can metabolize a similar number of solutes.

We have also estimated how many toxins gut bacteria can synthesize without being able to metabolize the same toxin and *vice versa*. Using this approach, we discover that *Brevundimonas sp., Campylobacter coli, Desulfovibrio sp., Oxalobacter formigenes, Campylobacter upsaliensis, Helicobacter pylori, Phascolarctobacterium faecium, Desulfovibrio piger* can synthesize more than 14 toxins without being able to metabolize the same toxins.

On the contrary, the following bacteria can only metabolize more than seven toxins without being able to synthesize them: *Pediococcus acidilactici, Listeria innocua, Listeria grayi, Lactobacillus ruminis, Klebsiella oxytoca, Clostridium sporogenes, Escherichia coli, Klebsiella sp., Enterococcus faecalis, Ruminococcaceae bacterium, Parvimonas micra.*

However, in general, KEGG pathways reaction direction is not strictly determined. Some KEGG reactions are included in separated "modules", where the reaction direction is given. Sadly, only 46 of 336 reactions are included in KEGG modules. We have added

information from the MetaCyc database, which contains more detailed information on reaction directions. Combining these two databases, we were able to strictly determine the direction of 151 from 336 enzymatic reactions, which are responsible for toxin synthesis/metabolism. Using this approach, we can more accurately identify the ability of bacteria to synthesize (without decomposing) or decompose (without synthesising) individual toxins. The most significant taxons of synthesizing and decomposing bacteria are given in Table 2, the full list is available in supplement table 3. Note that several bacterial taxa were included both in the list of toxin producers and in the list of toxin consumers. This apparent contradiction is explained by the fact that some species (genera) of bacteria can synthesize certain toxins, but at the same time consume (that is, remove) some other toxins. For example, *Pediococcus acidilactici* has metabolic pathways for the synthesis of homocysteine, indole-3-acetic acid, myoinositol, nicotinamide, y-guanidinobutyric acid, and at the same time, is able to consume creatinine, cytidine, methylglyoxal, S-adenosylhomocysteine, sorbitol, xanthosine. Specific uremic toxins synthesized/decomposed by a particular bacterium can be found in the supplement table 4.

Table 2. Bacteria determined as potential producers or consumers of some uremic toxins using analysis of KEGG modules with MetaCyc databases. Presented bacteria with more than five toxins producing (without decomposing the same toxin) or more than three toxins decomposing (without synthesising the same toxin). The full list is available in supplement table 3. Bacteria identified as both synthesizing and decomposing are presented in bold.

KEGG+MetaCyc	
Synthesis (of more than 5 toxins)	**Decomposition (of more than 4 toxins)**
Oxalobacter formigenes	***Escherichia sp.***
Stenotrophomonas sp.	***Rhizobium sp.***
Campylobacter coli	***Pediococcus acidilactici***
Brevundimonas sp.	*Lactobacillus ruminis*
Campylobacter upsaliensis	***Stenotrophomonas sp.***
Geobacter sp.	***Lactobacillus fermentum***
Escherichia sp.	*Lactobacillus paracasei*
Rhizobium sp.	*Lactobacillus casei*
Desulfovibrio sp.	*Lactobacillus rhamnosus*
Methylobacterium sp.	*Flavobacteriaceae bacterium*
Dialister pneumosintes	*Klebsiella sp.*
Helicobacter pylori	*Lactobacillus amylolyticus*
Desulfovibrio piger	***Corynebacterium ammoniagenes***
Edwardsiella tarda	*Clostridium sporogenes*
Morganella morganii	*Sphingomonas sp.*
Gordonibacter pamelaeae	*Hafnia alvei*
Clostridium sp.	*Escherichia coli*
Eggerthella lenta	*Providencia alcalifaciens*
Coprococcus catus	*Providencia rustigianii*
Pediococcus acidilactici	*Enterobacter cloacae*
Lactobacillus fermentum	*Listeria grayi*
Corynebacterium ammoniagenes	*Kocuria sp.*
Desulfitobacterium hafniense	*Lactobacillus acidophilus*
Paenisporosarcina sp.	*Pseudomonas sp.*

Paenibacillus sp.	*Klebsiella oxytoca*
Christensenella minuta	*Lactobacillus helveticus*
Aeromonas veronii	
Propionibacterium sp.	
Helicobacter bilis	
Phascolarctobacterium faecium	
Lachnospiraceae bacterium	
Helicobacter cinaedi	

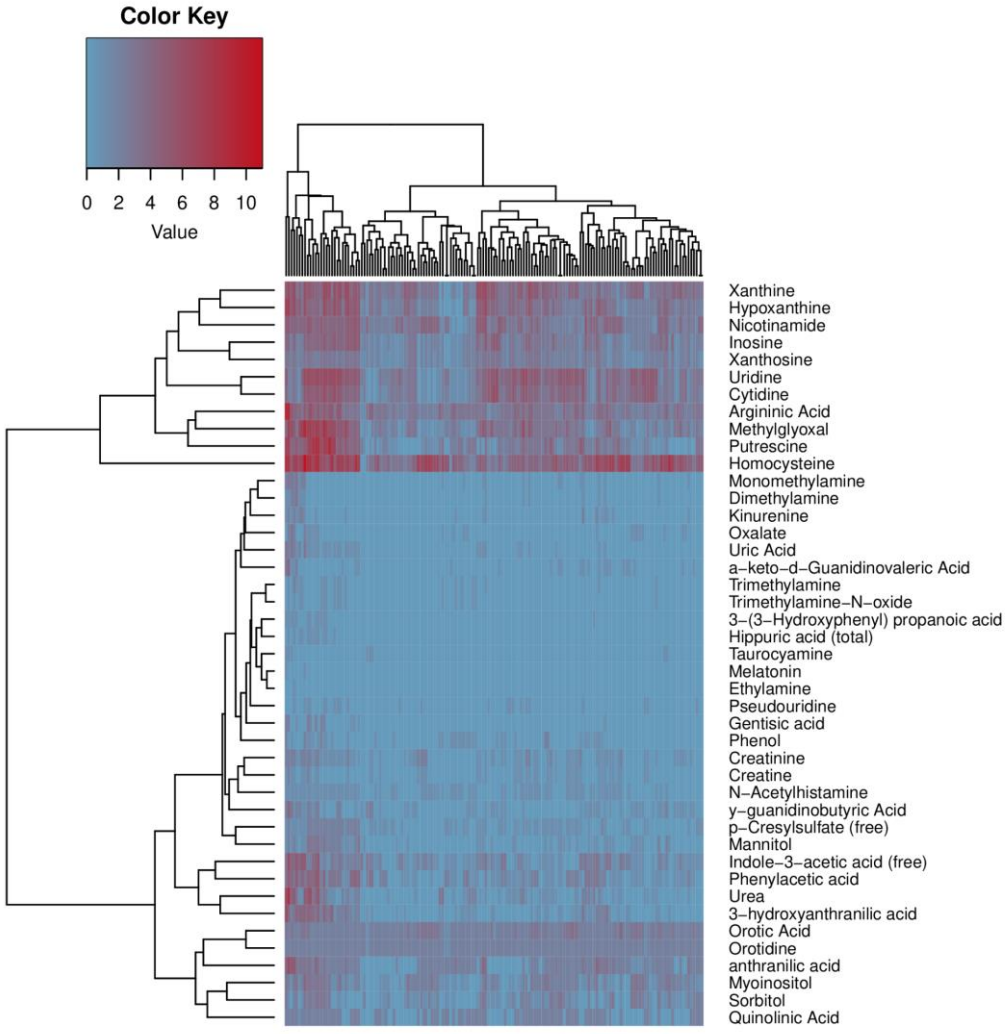


Figure 2: Cluster heatmap, which represents a clustering of toxins by bacteria that can potentially synthesize them. Color represents the number of enzymatic reactions in given bacteria that can potentially lead to the synthesis of a toxin (see Supplemental figure 1 for a high-resolution version with all bacteria names expanded).

3. Discussion

The main goal of this study was a bioinformatic analysis of the possible contribution of the intestinal microbiota to the synthesis of uremic toxins and the development of uremia in renal failure conditions. To date, several experimental studies demonstrated the possibility of such a link. We used several bioinformatic approaches to create a list of uremic toxins, which production is associated with the activity of various types of gut bacteria (Table 1). The relevance of the founded associations for each solute is discussed below.

Trimethylamine-N-oxide and closely related trimethylamine are well-known water-soluble uremic toxins. They are both considered to be microbiome-related [25]. Vanholder et al. estimated them as one of the most important toxins due to demonstrated experimental and clinical toxic effects [26]. We found 141 human gut microbial species that contribute to trimethylamine-N-oxide production via two enzymatic reactions. Moreover, there are no enzymes that can metabolize it, both in humans and in the microbiome. Besides renal failure, trimethylamine-N-oxide induces cardiovascular problems, stimulates up-regulation of a variety of macrophage scavenger receptors related to atherosclerosis development [27]. However, the exact mechanism by which trimethylamine-N-oxide accumulation leads to atherosclerosis is still unproven [28]. Moreover, there is a demonstration of a link between trimethylamine-N-oxide increased level and a risk of a heart attack [29]. Additionally, this compound is a suggested candidate mediating type-2 diabetes mellitus [30,31].

3-(3-Hydroxyphenyl) propanoic acid was earlier described as being produced by the microbiome [32] and associated with schizophrenia and autism [33]. In our study, 103 bacteria were identified as being able to produce this compound. Indeed, clinical observations in hemodialysis patients showed that this compound was reduced more than 10-fold in colectomy patients [15].

Another uremic toxin defined in our study as bacteria-derived, mannitol, is produced via a simple reaction in bacterial fructose and mannose metabolism. D-mannitol-1-phosphate phosphohydrolase catalyzes the hydrolysis of D-mannitol 1-phosphate to D-mannitol and phosphate. We found 227 gut bacteria species that conduct the reaction. In contrast to trimethylamine-N-oxide and pseudouridine, mannitol can be further metabolized by several bacterial enzymes. However, the ratio between its synthesis and metabolism is unknown. At the organism level, mannitol causes over-diuresis with the following dehydration [34]. Moreover, it demonstrates cytotoxicity to renal tubular epithelial cells, destroying cell cytoskeleton [35].

We identified five different specific bacterial enzymes that catalyze the production of phenol, uremic solute with well-documented negative effects in humans causing protein denaturation with the subsequent spreading necrosis [36]. Altogether, 192 gut bacterial species possess at least one of these enzymes. Phenol could be produced from tyrosine by microbial tyrosine phenol-lyase or may be synthesized from 4-hydroxybenzoate by 4-hydroxybenzoate decarboxylase or from catechol by phenol 2-monooxygenase that are parts of the aminobenzoate degradation pathway.

Oxalate is a toxin found to be enzymatically produced by bacteria, while humans synthesize it non-enzymatically. Oxalate is formed during purine metabolism when bacterial oxamate amidohydrolase catalyzes the transformation of oxamate to oxalate. We detected this reaction in 76 prokaryotic species from the human microbiome. As already mentioned, humans can produce oxalate non-enzymatically: for instance, ascorbic acid is metabolized in the human body with oxalate as an output [37]. Furthermore, significant amounts of oxalate enter the organism with food. The substance is known as the main component of kidney stones [38] and it modulates the immune system through an induced synthesis of cytokines, chemoattractants, and other inflammatory signal molecules causing degradation of IκBα in proximal tubular cells [39] and has an unfavorable impact on mitochondrial function [40].

Note, the enzymes identified by database-based approaches need for additional validation: oxalate and creatinine were attributed by this approach as “non-human

origin" toxins, while they are produced in human organism non-enzymatically, as well as pseudouridine, which is one of the main RNA catabolites [41–43].

In addition to toxins for which no potential synthetic pathways have been found in human metabolism, we showed for several toxins there are only 1-3 human enzymes that can potentially synthesize them. However, the question remains whether these enzymes contribute significantly to toxins synthesis, i.e. how many of these enzymes are there in the organism. We suggested that the optional way to evaluate enzyme abundance in the whole organism was to use the Human proteins atlas database with normalizing data of mRNA abundance on tissue weight. Understanding all the limitations of this approach, we believe that it can provide useful information. Thus, we estimated that some of these human toxin-producing enzymes presented at a reasonable level in human organism compared to others. Thus, we propose that these enzymes have a low contribution to uremic toxin synthesis and the role of microbiota predominates in the synthesis of corresponding substances. For example, orotidine concentration in the blood of uremic patients is quite high (1.20 (+/-1.60) mg/L), while the enzyme orotate phosphoribosyltransferase does not abound in the human organism (Fig. 1).

We have collected data on the potential of uremic toxins production for each bacterium, which can be found in the microbiome. The goal of such analysis was to identify "bad" and "good" bacteria in terms of uremic toxins production, i.e. to present putative targets for therapy of uremia. Of course, the fact that toxin can potentially be synthesized by gut bacteria does not mean that it inevitably enters the bloodstream, since bacteria, the same or others can consume it. Therefore, we have included the opportunity to metabolize toxins in our analysis as well. Predictably, usually, it is the same bacteria that synthesize the toxin since the solute is just an intermediate in some metabolic pathways. Theoretically, it is also possible to determine what bacteria can decompose some toxins, without being able to synthesize them; and *vice versa,* bacteria that can synthesize certain toxins, but don't have downstream metabolizing enzymes. Available databases do not include enough information for such analysis to be full-scaled. Nevertheless, our approach still gives some promising results. We defined *Desulfovibrio piger* as a potential "bad" bacterias synthesizing more toxins than they can degrade. Aronov and colleagues showed that *Desulfovibrio piger* is associated with a bad prognosis for uremia [14]. *Eggerthella lenta* was also a "bad", toxin-synthesizing bacteria in our analysis, and in parallel to our study, it was shown that this bacteria is associated with increased production of uremic toxins [44]. Another "bad" bacteria *Campylobacter upsaliensis*, which was one of the top "toxin-synthesizing bacteria" in our analysis, was associated with uremic syndrome [45]. In a case study, "bad" *Aeromonas veronii* was described as causing uremic syndrome [46]. Among "good" "toxin-decomposing" bacteria we revealed *Lactobacillus casei, Lactobacillus rhamnosus,* and *Lactobacillus acidophilus* which were earlier studied as a probiotic treatment for kidney failure [47–52]. *Helicobacter pylori,* which is conventionally considered to be malignant bacteria, was also among bacteria that can synthesize more toxins than consume, however, there are no direct links between it and kidney diseases [53].

Pediococcus acidilactici is a good example of the limitations of such analysis. In our analysis, it was both among synthesizing bacteria (it is able to synthesise 5 toxins without metabolizing them) and among toxin-metabolizing bacteria (6 toxins). As we mentioned above in the Results section, these are not the same toxins. The bacterium can synthesize homocysteine, indole-3-acetic acid, myoinositol, nicotinamide, y-guanidinobutyric acid and metabolize creatinine, cytidine, methylglyoxal, S-adenosylhomocysteine, sorbitol, and xanthosine. Moreover, it can both synthesize and decompose 9 more uremic toxins, which means these solutes are intermediates in some metabolic pathways. For *Pediococcus acidilactici* there is a clinical trial where it is included in probiotic treatment for uremic patients and thus it could be defined as "good" bacteria [54]. However, one can easily see from this example, that it is quite possible to miss valid targets due to databases scarcity or a very broad pull of accessible biochemical reactions for certain bacteria.

In an independent study, the total presence of *Klebsiella* and *Escherichia coli* significantly rises in the intestine of uremic patients [55], and we identified these bacteria among "metabolizing, but not synthesizing" of toxins. We can expect such bacteria will benefit from an increase in uremic toxins concentrations since they can utilize them during the revers transport to the intestine from the blood. However, it is hard to distinguish the causality: does the toxin rises, because bacteria produce it, and thus it is a "bad" bacteria, or bacteria amount rise because it can utilize the toxin and thus it is a "beneficial" one.

In this regard, it should be noted that bacteria can use uremic toxins as nutrients and thus eliminate hazardous solutes. For instance, some bacteria express urease (Pseudomonas spp.), the enzyme that catalyzes the hydrolysis of urea, or urate oxidase (Clostridia spp.), oxidizing uric acid [56]. Such toxic substances as oxalate and creatinine when releasing to the gut can be consequently metabolized by microbiota as well [57,58]: microbiota species from the genera *Oxalobacter*, *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Eubacterium* that are present in normal microbiota are capable to degrade oxalate, hence, diminishing its uremic accumulation [59]. According to our data, 39 bacterial species from the human microbiome conduct the compound breakdown.

Limitations

The main crucial limitation of this approach is databases completeness. Not all uremic toxins were found in KEGG, not for all of them enzymatic reactions were listed, not all reaction directions (synthesis or degradation) were given. However, most of these databases are constantly under annual update, and we hope that in the future it will be possible to include in such analysis even more important details, such as enzymatic reaction constants, bacteria abundance in the microbiome, and others.

4. Conclusions

We strongly suggest that usage of our data and approach would be not considered as solid evidence of some bacteria being beneficial or malignant in conditions of uremia, but rather to be a tool for new insights during experimental analysis. We believe that even in limited form these approaches can contribute to the treatment of uremia, suggesting new target bacteria and key enzymatic reactions.

5. Materials and Methods

Database usage

The Uremic toxins list was taken from the EUTox database and contained 134 compounds. Additionally, 12 compounds were added after the literature analysis.

Human Metabolome Database [22] was used to check the origins of toxins.

The human microbiome bacteria list was obtained from NIH Human Microbiome Project [60]. Different strains were collapsed into a single data for a bacterial species if possible. A list of 500 bacteria was obtained.

Human protein atlas was used to roughly estimate the abundance of enzymes in the human organism, using data on mRNA levels and average tissue weight.

MetaCyc database was used to manually check the directions of the reactions if it wasn't available in KEGG.

KEGG analysis

We analyzed 130 uremic toxins from The European Uremic Solutes Database (EUTox-DB) plus 12 from the literature analysis. For 97 toxins we found identifiers in the KEGG database (release: July 1, 2020) [61].

The receipt and processing of information obtained from the KEGG database were carried out using the R package KEGGREST (version 1.26.1) [62].

69 toxins were involved in any KEGG reaction, and 54 toxins were involved in enzymatic reactions for which the metabolic pathway is known.

482 bacteria were identified in KEGG for which the genus, species, and strain (or only genus and species) corresponded to the bacteria from the HMP list. All enzymes involved in all metabolic pathways described for each bacterium from the list and separately for human were obtained from the KEGG base.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1,

Figure S1: Expanded cluster heatmap, which represents a clustering of toxins by bacteria that can potentially synthesize them.

Table S1: All found connection toxin-reaction-bacteria

Table S2: Aggregated data on connection toxin-reaction-bacteria

Table S3: Full aggregated data on bacteria ability to synthesise or metabolize toxins

Table S4: List of toxins synthesised or metabolised by each bacteria

Author Contributions: Conceptualization E.Y.P.; methodology, A.A.Z.; software, A.A.Z.; validation, E.Y.P., E.A.D.; formal analysis, P.V.A., E.A.D., A.N.V.; writing—original draft preparation, P.V.A, E.A.D.; writing—review and editing P.V.A.; supervision, D.B.Z.; project administration, E.Y.P. All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest: The authors declare no conflict of interest.

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