Investigating the effects of urban input on the abundance and diversity of potential bio-floc forming bacteria in the River Murray, South Australia

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Abstract. Identifying members of the aquatic microbial community and their biotic and abiotic interactions are the first step in developing inocula for bio-floc starters used as aquaculture fish-feed. This research aims to identify whether a freshwater river with urban input can be used as a source of potential bio-floc forming bacteria. To identify the bacteria, 16S rDNA sequencing was performed, to determine the taxonomy and flow cytometry was employed to enumerate bacterial abundance. To resolve the complex interactions among microbes, microbial interaction networks were produced at the family level. Actinobacteria was found to be the most abundant bacterial phylum followed by Proteobacteria, Acidobacteria, and Chloroflexi that suggested the river was in health condition. Microbial interaction networks revealed nutrients, particularly nitrate, nitrite, ammonia, and silica, are crucial in maintaining network interactions, suggesting that urban nutrient input likely shapes the riverine microbial community. The families Actinomycetales F-ACK-M1, Rickettsiaceae, Betaproteobacteria O-SBl14 and Anaerolineae O-GCA004 demonstrated greatest network centrality, each interacting with seven first-neighbor taxa, suggesting an importance in community structure. Acetobacteraceae and Chloroflexi F-Dolo23 also exhibited network centrality and were directly linked to nitrate and nitrite, suggesting they play key roles in nitrogen cycling. Propionibacterium (44.82%) was the most dominant genera found in the Murray River followed by Anaerococcus (2.94%), and Finegoldia (2.05%). Comparison of the bacterial community comprising bio-floc and those found in the River Murray revealed that seven bacterial phyla including Proteobacteria, Bacteriodes, Cyanobacteria, Actinobacteria, Planctomycetes, Verrumicrobia, and Chloroflexi common to bio-floc contributed to 95.8% total relative abundance in the river. However, based on genera level, there were 14 bacteria genera in the river that generally present in bio-floc forming bacteria identified across all river samples constituted 4.63%. The potential bio-floc forming bacteria found mainly in downstream of the river provided various functions in the bio-floc system including producing natural flocculants to form microorganisms aggregates, a source of potential probiotics and prebiotics, nitrite oxidation and denitrification process, and degradation of organic matters.

Keywords: bio-floc forming bacteria, microbial interaction networks, aquaculture, riverine microbial ecology, River Murray
1. Introduction

In aquaculture systems, water quality from the aquatic environment needs to be optimized to support the growth of cultured fish. For a successful aquaculture endeavor, investigating the dynamics of water quality in the environment is essential, especially regarding the microbial community present, as bacteria can be harmful and beneficial for fish health [1]. The beneficial bacterial community is now recognized as a key component in aquacultural bio-floc technology [2]. Bio-floc technology is an alternative fish-feed composed of a variety of microorganisms, mainly bacteria in symbiotic mutualism with microalgae, protozoa, rotifers, nematodes and organic matter aggregates [3]. Because of these beneficial microbes, bio-floc is an excellent source of microbial proteins and nutrients that are directly accessible to cultured fish [4]. The use of bio-floc technology as a natural aquafeed reduces cost input and improves aquaculture production [1]. Therefore, bio-floc technology is a promising and important innovation in aquaculture which decreases the use of commercial feed, contributing to 40-60% of total aquaculture production [5]. The commercial use of microbial bio-floc has been implemented successfully in the shrimp farming industries of Belize, Indonesia, and Malaysia [6], and has significantly increased the production of farmed Tilapia in China [7]. Yun et al. [8] reported that there are six bacterial phyla commonly found in freshwater developed bio-floc, the Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria, Actinobacteria, Bacilli, and Bacteroidetes, while specific bacteria include the Acidovorax, Aeromonas, Agrobacterium, Bacillus, and Rhodococcus. Therefore, the presence of these taxa is likely essential for the development of freshwater aquaculture-based bio-floc technology in South Australia.

Identifying members of the microbial community in the aquatic environment is the first step in developing inocula for bio-floc starters [1]. The growth of bio-floc bacteria in aquaculture systems depends on carbon and nitrogen (C/N) contained in organic matter to provide an optimum C/N ratio of more than 10:1 [9]. The impact of organic matter on bacterial composition has been identified in the River Murray, with studies suggesting that bacterial abundance is closely related to the concentration of organic matter present, influenced by human input of organics into the river [10]. Ikbeke et al. [11] reported that in freshwater ecosystems bacterial diversity was dominated by Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria and the bacterial community showed a different composition between sites of agricultural and urban inputs. Due to the high nutrient demand of bio-floc forming bacteria, it was hypothesized that potential bio-floc forming bacteria will be dominant in areas where human input is large. Thus, research was conducted in two locations along the Murray River, upstream and downstream of Murray Bridge, a rural town in South Australia that resides on the river’s bank, to identify whether urban input can be used as a key to find potential bio-floc forming bacteria. It was predicted that microbial abundance and diversity would increase downstream, as a result of anthropogenic nutrients run-off from Murray Bridge. Bacterial composition upstream and downstream from Murray Bridge were identified via 16s rDNA sequencing and computational analyses. The bacterial candidates sought were those which commonly form bio-floc. The biotic and abiotic interactions among microbes and their environment were resolved based on microbial interaction networks.

2. Materials and methods

2.1 Sampling and data collection

The study was conducted upstream in Mobilong (35°6’18”S, 139°16’30”E) and 3.3 km downstream in Long Island Reserve (35°87’40”S,139°817’42”E), South Australia. Triplicate water samples of 5L were collected from the river and analyzed to determine nutrient concentrations and the bacterial communities present. Nutrients were analyzed in water samples via an LF 2400 photometer using aquaslex included nitrate, nitrite, ammonium, silica, iron, and chlorine. The taxonomic composition of bacteria was examined via 16s rDNA sequencing and computational taxonomic analyses, while microbial abundance was determined by flow cytometry [12]. Microbial taxonomy commonly forming bio-floc was obtained from the study of Cardona et al. [4], who investigated bio-floc forming bacteria in shrimp culture ponds based on phyla level and the abundance of bacteria present in bio-floc was obtained from a study on shrimp culture in circular tanks by Rajkumar et al. [13]. To identify bio-floc
forming bacteria-based genera, some literatures in bio-floc research were studied to compare bacteria found in the river with bio-floc system.

2.2. **DNA sequencing and taxonomic analysis**

To determine the taxonomic composition of bacteria upstream and downstream of Murray Bridge, 16s rDNA sequencing was performed. Five-liter water samples taken in triplicate were filtered through 5 μm filters (Whatman) followed by 0.22 μm MoBio filters to collect bacteria. A MoBio PowerWater DNA isolation kit was used to extract bacterial DNA, and DNA concentration determined via a Qubit fluorometer. Polymerase chain reaction (PCR) was implemented to amplify the 16s rDNA gene and amplicons were purified by Agencourt Ampure beads (Agencourt Bioscience Corporation, Beverly, MA, USA). Amplicons were then sequenced by Molecular Research LP, Shallowater TX, USA and subsequent FASTA and QUAL files produced. These files were converted to FASTQ and operational taxonomic units (OTU) picked using the QIIME pipeline, encompassing USEARCH, UPARSE, UCHIME and Python scripts to create OTU tables [12].

2.3. **Flow cytometry**

Bacterial and viral abundance was determined by flow cytometry based on procedure conducted by Dann et al. [14]. Triplicate water samples (1 mL) were transferred to 2 mL cryo vials containing 20 μL glutaraldehyde (0.5% concentration). Samples were stored in the dark at 4°C for 15 minutes, snap-frozen in liquid nitrogen and stored at -80°C. Samples were then diluted in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA) and stained with SYBR green I.1 μm fluorescent beads (Molecular Probes) were added to each sample as a size and concentration standard. Samples were run on a FACSCanto II flow cytometer. To determine bacterial and viral counts, biparametric plots of SYBR Green fluorescence (nucleic acid content) and side scatter (cell size) were analyzed as performed previously.

2.4. **Taxonomic comparison and network analysis**

The taxonomic composition of bacteria upstream and downstream of Murray Bridge was compared at the phyla and family levels. One-way analysis of variance (ANOVA) was performed using SPSS 16.0 to compare the difference in bacterial abundance of upstream sites, downstream sites, and bio-floc. Significant differences were confirmed when a 5% significance level (p = < 0.05) was reached. To determine and visualize the multitude of interactions among microorganisms, network analyses were implemented at the family level. Taxa abundance and meta data with missing values or zeroes were replaced as Na N and any taxa with missing values in more than three samples were removed to reduce erroneous analysis in Co Net. The filtered data was converted to a tab-delimited text file and imported into the Co Net application embedded in Cytoscape 3.6.1. Pearson and Spearman thresholds were set at a significance level of 0.05, while distance parameters were used to filter data via Bray Curtis dissimilarity using a 0.1 significance level followed by randomization. The importance of taxa in network structure was determined based on centrality measures of the nodes, including average shortest path length, betweenness centrality, and closeness centrality, as well as the edge interactions of positive degrees and edge betweenness. The average shortest path length (AL) is defined as the average length of shortest paths possible between two nodes in the network [15]. Betweenness centrality (BC) represents how many times a node is used to connect the shortest path between two other nodes [16], and closeness centrality (CC) calculates the average distance of a node to another node based on all nodes in the network [17].

3. **Results**

**3.1. Bacterial community composition**

Bacterial community composition at the phyla and family levels present upstream and downstream of Murray Bridge in the River Murray are presented in Figure 1.A total of 29 bacterial phyla excluding unassigned taxa were found in the River Murray (Figure 1.A) and 164 families (Figure 1.B). The bacterial composition presented similar patterns between upstream and downstream sampling locations with Actinobacteria being the most abundant phylum followed by Proteobacteria, Acidobacteria, and Chloroflexi. However, a starkly different composition occurred in the first
sampling location downstream. Here, Actinobacteria was still the most abundant, however, it was then followed by Firmicutes, Cyanobacteria, and Proteobacteria. Actinobacteria dominated the microbial community both upstream and downstream of the river, varying from 30.75% to 51.86% total relative abundance across samples. Proteobacteria was the second abundant ranging from 8.30% to 46.78% followed by Acidobacteria and Chloroflexi varying from 4.08% to 7.84% and 2.23% to 5.17% total relative abundance across samples respectively. In upstream 1, Actinobacteria and Proteobacteria constituted 82.24% total abundance and tended to decrease in upstream 2 and upstream 3 representing 79.58% and 75.74% total relative abundance respectively. These bacteria further decreased in abundance in downstream 1, representing 60% total relative abundance, as the abundance of other taxa such as Firmicutes and Cyanobacteria increased. However, Actinobacteria and Proteobacteria were significantly greater in downstream 2 and downstream 3, representing 77.53% and 82.82% total relative abundance respectively.

Presented in the legends are all (29) bacterial phyla and the 20 most dominant families across all samples. Different taxonomic groups are shown by distinct color sin the bar chart. Family level taxa are represented by lowest taxonomic rank determined in QIIME. The letters before names indicate lowest taxonomic rank IDs where C=Class, O =Order, and F = family.

A clear difference in the taxonomic composition was seen in downstream 1 in comparison to the two other downstream samples (Figure 1.A.) In the downstream 1 sample, bacterial abundance was dominated by Propionibacteriaceae and Streptophyta, which totalled 58.42% total relative abundance, while in the remaining downstream samples, Actinomycetales ACK-M1 was the most abundant, varying from 3.65% to 41.35% followed by Rickettsiaceae and Holophagaceae which varied from 3.68% to 30.31% and 0.35% to 6.13% respectively.

3.2. Microbial interaction network
The microbial interaction network resolved the biotic and abiotic relationships between microbial families inhabiting the River Murray and environmental variables, such as nutrient presence and concentration. The network divided into two groups consisting of 43 families, represented by 43 nodes and 76 interactions (edges) (Figure 2). The network was obtained by filtering 109 families and 2013 interactions using The Bray-Curtis dissimilarity index at a threshold of 0.1.
Figure 2. Microbial interaction network at the family level upstream and downstream of Murray Bridge in the River Murray, South Australia.

The two networks present are ordered circularly via average shortest path length and separated by the presence of environmental variables including nitrate, nitrite, ammonia, and silica. Node size and color indicate relative abundance and betweenness centrality, and edge width and color represent edge weight and edge betweenness respectively. The network was created using Cytoscape v3.6.1 with the embedded CoNet application. Networks were filtered by a Bray-Curtis dissimilarity index threshold of 0.1 and the correlation parameters analyzed using Pearson and Spearman with a P-value threshold of 0.05.

The four most dominant families, including ActinomycetalesF-ACK-M1, Rickettsiaceae, BetaproteobacteriaO-SBlal4 and AnaerolineaeO-GCA004 were present in the first network and demonstrated complex interactions when compared to the other families. Actinomycetales ACK-M1 was the most abundant family in the networks, indicated by the large node size. This family exhibited extensive network centrality, having seven families as first neighbours and AL, BC, and CC values of 2.055, 0.177, and 0.486 respectively. The other families present had a similar interaction pattern to Actinomycetales ACK-M1, including Rickettsiaceae (AL 2.166, BC 0.2108, and CC 0.4615), Betaproteobacteria O-SBlal4 (AL 2.0, BC 0.2599, and CC 0.5) and Anaerolineae O-GCA004 (AL 1.889, BC 0.2345, and CC 0.5294). Due to the clustering of nutrients, the network presented to the right provides evidence of the bacterial families that are influenced by changing nutrient levels. In this network, Acetobacteraceae and ChloroflexiF-Dolo23 show more complex interactions compared to the other microbes. Acetobacteraceae exhibited connections to four families including Marinicellaceae, Weeksellaceae, ChloroflexiF-Dolo23 and Nitrosomonaceae, as well as having nitrate as a first neighbor. The edge betweenness between nitrate and Acetobacteraceae was 60 and the greatest in the network. ChloroflexiF-Dolo23 also interacted with four bacterial families including Ellin675, Weeksellaceae, Cerasiococcaceae, and Acetobacteraceae, as well as a direct link to Nitrite. Nitrite itself shares interactions with other microbial families including Holophagaceae and Cerasiococcaceae. The greatest edge betweenness (16.67) was present between Nitrite and Holophagaceae.

3.3. Comparison of bacterial communities present in bio-floc and the River Murray
Comparing the bacterial communities upstream and downstream of Murray Bridge and those identified in bio-floc by Cardona et al. shows that seven bacterial phyla dominating bio-floc including...
Proteobacteria, Bacteroidetes, Cyanobacteria, Actinobacteria, Planctomycetes, Verrumibrobia, and Chloroflexi are also found in the River Murray (Figure 3) [4]. The seven bio-floc forming bacterial phyla identified in the River Murray contribute 95.80% of the relative bacterial abundance identified in bio-floc. These same seven taxa contribute 86.93% and 83.5% of the total relative abundance upstream and downstream of Murray bridge respectively. Proteobacteria was the most abundant phyla in bio-floc samples (60.07%), while Actinobacteria was the most dominant phyla upstream (42.65%) and downstream (41.11%) of Murray Bridge. The second most abundant bacterial phyla in bio-floc is Bacteroidetes (21.86%) followed by Cyanobacteria (8.48%) and Actinobacteria (2.13%), while in the River Murray, Proteobacteria was the second most abundant, constituting 36.54% and 32.39% total relative abundance in upstream and downstream samples respectively, followed by Chloroflexi and Bacteroides.

![Figure 3](image)

Figure 3. Comparison of bacterial composition between the River Murray and bio-floc at the phyla level

The bio-floc forming bacterial composition data was acquired from Cardona et al. [4]. Seven bacterial phyla contributing 95.80% bio-floc forming bacteria and present in high abundance in the River Murray are presented in the legend. Bacterial composition data presented in upstream and downstream are means of bacterial abundance from three sampling locations. A comparison of bacterial abundance upstream and downstream of Murray Bridge and in bio-floc presented by Rajkumar et al. (Figure 4) [13]. Bacteria in bio-floc are more abundant than were determined in the River Murray. Bacterial abundance was on average greater downstream than upstream of Murray Bridge, ranging from $4.22 \times 10^7$ cells/ml to $6.22 \times 10^7$ cells/ml and $2.85 \times 10^7$ cells/ml to $4.43 \times 10^7$ cells/ml respectively. ANOVA analysis suggested a significant difference of bacterial abundance between bio-floc, upstream and downstream samples ($p=0.00$, $p<0.05$). A post hoc test (Bonferroni) was conducted to determine significant differences between two sampling locations. A significant difference between bio-floc and upstream ($p=0.001$, $p<0.05$) was present, as well as bio-floc and downstream ($p=0.002$, $p<0.05$), while there was no significant difference in bacterial abundance of upstream and downstream samples ($p=0.525$, $p>0.05$).
Figure 4. Comparison of bacterial abundance between upstream and downstream of Murray Bridge along the River Murray and bacteria in bio-floc. The bio-floc forming bacterial abundance data was obtained from Rajkumar et al. [13]

We identified about 175 bacterial genera (65%) excluding unassigned taxa (35%) detected in upstream and downstream of the river (Figure 5). The 20 most dominant bacteria contributed to 60% abundance, while there were 155 genera constituted 5% abundance. Propionibacterium (44.82%) was the most dominant genus found in the Murray River followed by Anaerococcus (2.94%), and Finegoldia (2.05%). There were 14 genera that presented potential bio-floc forming bacteria identified across all river samples constituted 4.63% (Table 1). The genera mainly presented in downstream of the river, while five genera were found in upstream areas. The potential bio-floc forming bacteria found in the river provided various functions in the bio-floc system as presented in table 1. Streptococcus (1.92%) is the most dominant bio-floc forming bacteria followed by Acinetobacter (0.84%) and Staphylococcus (0.77%).
Figure 5. The relative abundance of the 20 most dominant genera constituted 60% of the total 175 bacteria across all river samples excluding unassigned taxa (35%).

Table 1. Potential bio-floc forming bacteria found in upstream and downstream of the River Murray provided various functions in the bio-floc system.

| No | Function                              | Genus/Species    | Relative abundance (%) | Phylum            | Reference |
|----|---------------------------------------|------------------|------------------------|-------------------|-----------|
| 1  | Producing natural flocculant to create microorganisms aggregates | Streptococcus    | 1.92                   | Firmicutes        | [18]      |
|    |                                       | Staphylococcus   | 0.77                   |                   |           |
|    |                                       | Bacillus         | 0.009                  |                   |           |
|    |                                       | Neisseria sp.    | 0.008                  | Proteobacteria    | [19]      |
| 2  | A source of potential probiotics      | Bacillus sp.     | 0.05                   | Firmicutes        | [20]      |
|    |                                       | Lactococcus      |                        |                   | [2]       |
|    |                                       | Rhodococcus      | 0.02                   | Actinobacteria    | [21]      |
|    |                                       | Kocuria          | 0.008                  |                   |           |
| 3  | A source of potential prebiotic        | Pseudomonas      | 0.76                   | Proteobacteria    | [20]      |
| 4  | Nitrification and denitrification process | Nitrospira     | 0.15                   | Nitrospirae       | [22]      |
|    |                                       | Rhodobacter      | 0.02                   | Proteobacteria    | [23]      |
| 5  | Degradation of organic matters         | Sphingomonas     | 0.006                  | Proteobacteria    | [19]      |
|    |                                       | Burkholderia    | 0.02                   |                   | [24]      |
|    |                                       | Acinetobacter    | 0.84                   |                   |           |
|    | Total                                 |                  | 4.63                   |                   |           |
4. Discussion

4.1. Taxonomic composition and abundance

Investigating the taxonomic composition and abundance of microorganisms inhabiting the River Murray is an essential step in determining whether bacteria are present that can potentially be used in aquacultural bio-floc. In this study, we found that Actinobacteria were the most dominant within the river. This result was relevant to previous studies conducted in various freshwater locations that also showed more than 50% Actinobacteria comprise the total bacterial community abundance [25]. The domination of Actinobacteria in freshwater systems likely generates beneficial impacts on river water quality, as they have been demonstrated to degrade complex mixtures of organic matter [26]. These photoheterotrophic bacteria are also nutritionally efficient, capable of adapting to oligotrophic conditions [27]. Therefore, when Actinobacteria are present in large abundance, it is likely that cyanobacterial abundance is low, as they require eutrophic conditions to support their growth. This was supported by observations here, where cyanobacterial abundance increased in the downstream sample from Murray Bridge, likely due to increased nutrient concentrations in the water from urban input. Thus, while the presence of cyanobacteria is commonly used as an indicator of damage to aquatic ecosystems, such as eutrophication, the occurrence of Actinobacteria can be considered as sentinels from deterioration of the aquatic environment [26]. Proteobacteria was the second most abundant phyla in the river. The occurrence of proteobacteria is commonly related to changes in concentrations of nutrients and organic matter. A significant decrease of proteobacterial abundance was identified in the downstream sample, likely due to an increase in nutrients and organic matter as a result of urban input. This inference is supported by the research of Magalhaes et al. [28], who revealed that changing environmental conditions impacts the abundance of Proteobacteria in the freshwater environment. Decreasing proteobacterial abundance led to an increase in abundance of Firmicutes and Cyanobacteria in the downstream sample. The growth of Firmicutes is a significant positive correlation with dissolved organic carbon and turbidity [29], and this phyla is expected to be present in bio-floc, as the genera *Streptococcus*, *Staphylococcus*, *Lactobacillus*, and *Bacillus* are commonly used to create microorganisms aggregates [18]. The presence of Proteobacteria and Firmicutes likely are the main source of the bio-floc forming bacteria as they involved in multiple essential functions in bio-floc aquaculture systems (Table 1, Additional information). The Acidobacteria are another phyla capable of adapting to various changes in environmental conditions. Most Acidobacteria are heterotrophs that use nitrite as a nitrogen source and micronutrients in oligotrophic condition [30]. At the family level, the trend in bacterial abundance and composition followed that of the phyla level. Actinomycetales ACK-M1 (Actinobacteria) was the most abundant followed by Rickettsiaceae (Proteobacteria) and Holophagaceae (Acidobacteria). A significant change in bacterial composition occurred in the downstream sample where urban input from Murray Bridge was presumed great. Here, Propionibacteriaceae (Actinobacteria) was the most abundant followed by Streptophyta (Cyanobacteria). The microbial abundance downstream of Murray Bridge was greater than sites upstream ranging from $4.22 \times 10^7$ cells/ml to $6.22 \times 10^7$ cells/ml and $2.85 \times 10^7$ cells/ml to $4.43 \times 10^7$ cells/ml respectively. This suggests that the microbial abundance and diversity increased downstream, as a result of anthropogenic land run-off from Murray Bridge.

4.2. Interactions among microbes and environmental variables

The microbial interaction network resolved the complex interactions among microbes and their abiotic environment for bacterial families inhabiting the River Murray. The two networks produced were ordered circularly via average shortest pathlength and separated by the presence of environmental variables including nitrate, nitrite, ammonia, and silica (Figure 2). These networks show that microorganisms do not function independently, but rather they form complex ecological interactions, where microbes are interdependent [31]. Due to nutrient clustering in the alternate network, the first network shows the microorganisms whose interactions are likely not affected greatly by the presence and concentrations of nutrients. Actinomycetales F-ACK-M1, Rickettsiaceae, Betaproteobacteria OSBL14 and Anaerolineae O-GCA004 demonstrated more complex links compared to the other microbes, as they had seven families as first neighbours. Co-occurrence and relationship patterns among microorganisms can be positive, negative or neutral interactions [15]. Actinomycetales F-
ACK-M1 and Rickettsiaceae were more abundant than other families, while the remaining families showed similar abundances to one another. As the interaction between two microorganisms shows a similar abundance pattern than other microbes, these conditions are possibly predicted having a positive relationship that occurred because of cross-feeding, co-aggregation in biofilms, co-colonization, niche overlap or other factors, while once microorganisms dominate to other or the species increase its abundance that lead to decreasing or disappearing other species, a negative relationship is assumed as the result from amensalism, a prey-predator relationship, competition, and other reasons [31]. The second network presented to the right shows the clustering of nutrients, providing evidence of the bacterial families that are likely influenced by changing nutrient levels in the river. As bacterial co-occurrence patterns are influenced by environmental factors, this suggests that these bacterial families require a particular environmental parameter for optimal growth, or that they function to transform nutrients [32]. Acetobacteraceae exhibited interactions with four families, and had nitrate as a first neighbor, while Chloroflexi F-Dolo23 also interacted with four bacterial families, as well as having a direct link to nitrite. The strong interactions between bacterial families and nutrients presented here suggests the bacteria may play a role in biogeochemical cycling [33].

4.3. Potential bio-floc forming bacteria composition

Seven bacterial phyla are present in great abundance in the river including Proteobacteria, Bacteriodetes, Cyanobacteria, Actinobacteria, Planctomycetes, Verrumicrobia, and Chloroflexi, contributed to 95.80% of bacterial abundance found in bio-flocs. However, based on genera level there were about 4.63% abundance of bacterial genera in the river present in bio-floc forming bacteria composition. In bio-floc system, bacteria are cultured from targeted species, feed, and carbohydrate source [34]. The targeted bacteria forming bio-floc system normally provided different rules, for instant, Bacillus and Lactococcus produce a natural flocculant used to produce microorganisms aggregates and a source of potential probiotics [2, 18, 20] Burkholderia and Sphingomonas play an important role in degradative organic matters [19]. To create a bio-flocsystem, water is fertilized using organic matter and nitrogenous compounds, allowing bacteria to grow exponentially, resulting in the co-aggregation of microbes required in bio-floc formation [4]. This indicates that bio-floc forming bacteria require eutrophic conditions rather than oligotrophic waters for optimal growth. Bio-floc forming bacterial abundance showed a significant difference compared to the bacterial abundance inhabiting the River Murray. This was likely the case because organic carbon and nutrients were provided in an optimum 10:1 ratio, allowing bacteria to thrive in the bio-floc system [9]. Propionibacterium was dominant genus found in the river contributed 44.82% abundance. Although this genus was not included in the bio-floc forming bacteria, the bacteria was categorized as a beneficial microbe that contained B group vitamins including B12, trehalose, and various bacteriocins are potential used in the food, cosmetic, and pharmaceutical sectors [35]. The other beneficial bacteria were Corynebacterium commonly used in the medical, veterinary, and biotechnology industries [36].

5. Conclusion

Urban input influenced the abundance and diversity of potential bio-floc forming bacteria in the River Murray. This was observed in the bacterial abundance and composition of the first sampling location downstream, where the urban input was presumed great in comparison to upstream sampling sites. The effect of environmental factors on microorganisms was demonstrated in the microbial interaction network between microbes and nutrient metadata. The two networks produced were separated by the presence of environmental variables including nitrate, nitrite, ammonia, and silica. This indicated that the abundance of certain microbes was strongly associated with changing nutrient concentrations. It is relevant with requirement in producing of a bio-floc system. Water commonly is fertilized by organic matter and nitrogenous compounds for optimal growth of certain bacteria resulting in the co-aggregation of microbes required in bio-floc formation. The potential bio-floc forming bacteria found in the river provided various functions in the bio-floc system. To better understand whether the potential bio-floc forming bacteria can be used for bio-floc, future research should be undertaken, focusing on isolation and culture of bio-floc specific genera and species.
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