Obtainment of Lignocellulose Degradation Microbial Community: The Effect of Acid-Base Combination After Restrictive Enrichment

Binbin Hua (huabb@nankai.edu.cn)
Nankai University https://orcid.org/0000-0002-5781-5900
Xiaofen Wang
China Agricultural University https://orcid.org/0000-0003-3543-1073
Zongjun Cui
China Agricultural University

Research Article

Keywords: acid-base combination, restrictive enrichment, lignocellulose, microbial community

Posted Date: November 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1074700/v1

Abstract

Acid-base combination is used in some cases expecially after restricted enrichment, and has created many lignocellulose-degrading communities. While how it worked is not well understood. In this study, compost was used as inoculum source. Induced community structure changes were analyzed with high throughput sequencing to elucidate the formation processes and determine the mechanisms of acid-base combination. We found that after restricted enrichment, retaining primarily bacteria not only included that could decompose and utilize lignocellulose, such as *Clostridium* and *Pseudomonas*, but also synergistic microbiota such as *Pseudoxanomonas* and *Alkalobacillaceae.* When the proportion of these two types of bacteria was not balanced, the degradation ability of the microbial community was low or pH changes of it did not compound regular changes , which maybe lead to the failure of restricted enrichment. Microbial communities were re-constituted by acid-base combination, whereby the degrading and synergistic strains were adjusted to a more appropriate proportion. Acid-base combination fixed the instability of microbial communities caused by randomness of restrictive screening enrichment. In this study, the mechanism of acid-base combination was analyzed, which enriched the theoretical system of restricted culture, and provided an effective and controllable technical method for obtaining high-quality lignocellulose-degrading microbial community resources.

Introduction

In nature, lignocellulose degradation often requires various microorganisms to cooperate as a microbial consortium (Tesfaw and Assefa, 2014; Wang et al., 2020). Inspired by natural microbial consortia, artificial microbial consortia can be created to address questions in scientific research and problems in industrial production (Xu and Yu, 2021). Nevertheless, simple artificial consortia suffer from the shortage of characterized strains for synthetic consortia construction and the inability to co-culture microorganisms stably (Weiland, 2010). In contrast, undefined natural consortia originate from environmental microbial communities with an unknown number of constituents, often with outstanding self-stability and low operation requirements (Jawed et al., 2019). Given the advantages of restrictive screen enrichment, such as reduced metabolic burden and robustness to environment disturbances, many lignocellulose-degrading microbial communities are enriched by this process (Cui et al., 2002; Hui et al., 2013; S. et al., 2002; Wang et al., 2011).

Despite these benefits, however, the uncontrollability of enrichment culturing requires many parallel experiments in order to obtain microbial communities with high lignocellulose degradability. Further, often the degradability of initially suitable communities can decrease in subsequent successive transferring. To overcome these challenges, acid-base combination of microbial community was used in the restrictive screen process (S. et al., 2002). For acid-base variety, one microbial community with low pH was combined with another microbial community with high pH to obtain a novel community whose pH fluctuated within a specific range. With the method of restricted culture and acid-base combination, many lignocellulose-degrading microbial communities, such as MC1 (S. et al., 2002), XDC-2(Guo et al., 2010), and WCS-6 (Wang et al., 2011), were obtained. MC1, for example, was constructed from compost and is capable of effectively degrading various cellulosic materials, including rice straw (Cui et al., 2002; Hua et al., 2014), filter paper (Kato et al., 2004), cardboard (Yuan et al., 2012). In addition, it has been used for hydrolysis and acidification of agricultural waste (Yu et al., 2017), to enhance anaerobic digestion (Hua et al., 2016; Yuan et al., 2016), for concurrent saccharification and anaerobic digestion of Napier grass (Wen et al., 2015). After more than 40 successive generations of culture (S. et al., 2002) and approximately 20 years of research and application (Wang et al., 2021), the microbial community has remained stable with a high degradation efficiency. Therefore, in terms of practical application, restrictive screen enrichment and acid-base combination have achieved good results. At the same time, however, little research has been conducted into understanding the mechanisms of acid-base

combinations. It is currently unknown how these communities form, particularly how acid-base combinations influence the microbial communities. With recent advances in sequencing, high throughput approaches have made it possible to quantify the composition of microbial communities during this process (Estrela et al., 2021) .

This study used compost as the inoculum source for the restrictive screen lignocellulosic degradation microbial community. The community structure changes were analyzed by high throughput sequencing to understand the formation process and reveal the mechanisms of acid-base combinations after long-time enrichment cultures.

Materials And Methods

Inoculation and enrichment culturing

Our inoculum source was compost, sourced from the Zhuozhou Experimental Station, China Agricultural University. The pile was turned over once every two days during the thermophilic period (average 60 ℃) and once every five days after the temperature dropped to 35℃. Overall, the experiment lasted for 60 days.

Inoculum sources (5g in fresh weight) were sampled during the ripening period (D1) and thermophilic period (D2). These were then inoculated into PCS medium with three replicates (A1, A2, A3 and B1, B2, B3, respectively). Cultures were maintained in 100 mL triangular bottles for 15 days in each generation.

The PCS medium was 0.5% peptone, 1 % lignocellulose, 0.5% NaCl, 0.2% $CaCO_3$, 0.1% yeast powder, pH natural (about 7.3) (S. et al., 2002). Rice straw was utilised as a carbon source, and small filter paper strips were added to the cultures. Those with decomposed filter paper strips were regarded as degradable and were chosen for the succession cultures. The inoculation was performed statically at 50 ℃ at a volume ratio of 5% (v/v).

Pretreatment of lignocellulosic materials and analysis of weight loss

The rice straw (harvested from Shangzhuang Experimental Station of China Agricultural University) was cut into approximately 10 cm length, soaked in 1% NaOH for 24 h, and washed with tap water until the pH value was between 7.0 and 8.0. Pieces were then dried at 50℃ and stored for future use. The weight loss of rice straw was tested as previously reported (Hua et al., 2014).

Acid-base combination of microbial community

Dynamic changes in pH were measured, communities with a slight pH decrease and a slow pH increase were selected. 5 mL liquid of each community was extracted, taken, and combined to create a novel PCS medium. After merging, these new communities were named with the original group numbers (A1A3, A1B3, A3, and B2B3), as shown in Fig 1.

Extraction of genomic DNA

After five months of restrictive screening, samples were extracted from D1, D2, A1, A2, A3, B1, B2, B3, respectively, and after acid-base combination, samples were also extracted from A1A3, A1B3, A3B2, and B2B3, respectively. Genomic DNA was extracted using the CTAB method (Murray and Thompson, 1980).

High-throughput sequencing analysis

Genomic DNA was sent off for high throughput sequencing (Allwegene Tech., Co., Ltd, Beijing). The V3+V4 region was amplified using the following universal primer sets: 336 F and 806 R (5'-GTACTCCTACGGG AGGCAGCA-3'and 5'-GTGGACTACHVGG GTWTCTAAT-3'). The amplicon libraries were sequenced using the Illumina Miseq and a 2×300 bp paired-end protocol (Bartram et al., 2011).

Sequences were filtered out with a quality value <20. For each sample, the qualified reads were defined as a library and then down-sampled to the smallest library size, which was determined to compare the alpha diversity metrics, including Chao and Shannon (mothur v.1.30.1). After demultiplexing, the reads were assigned species-equivalent operational taxonomic units (OTUs) at 97% sequence similarity. The remaining OTUs were denominated at different classification levels according to the Silva database release 115 (http://www.arb-silva.de).

Results

Lignocellulosic degradation ability of the obtained microbial communities.

The microbial community A1 had the lowest weight loss of rice straw (13.91±4.83%), followed by community B2 (38.28±1.77%). Community B1, A2, B3 and A3 increased successively (42.77±2.86%, 46.03±6.96%, 46.28±3.43%, and 47.08±0.40%) (Fig. 2A). These values corresponded to changes in pH.

Decreases in pH reflected rapid decomposition of cellulose, while increases in pH occurred with decreasing rates or cessation of cellulose degradation (Liu et al., 2006). Solutions' pH decreases were a prominent feature of the cellulosic materials degraded microbial community (Liu et al., 2006). The pH of all six microbial communities decreased on the third day Fig. 2C. The pH of A1 and B2 decreased to 6.6 and then began to rise rapidly to alkaline conditions, while A2 and B1 decreased to approximately 6.3. On the third day, A3 and B3 decreased to 6.1 and 6.3, respectively, and remained acidic until the 12th day. On the 15th day, the pH of A3 returned to neutral, while the pH of B3 remained acidic.

Microbial degradation of straw resulted in pH decreases in all treatments before rising to slightly alkaline conditions. Although A3 and B3 displayed faster degradation rates, the recovery time to slightly alkaline was longer than 12 days. Therefore, we selected B1 and A2 for further research and utilization. For A3 and B3, as the acidic products were not rapidly utilized, accumulation of these products may inhibit the degrading strains, thus inducing instability of the bacterial communities.

To study the acid-base combinations, A1 and B3 were pairwise combined with A3 and B3, respectively (Fig. 1). The new microbial communities A1A3, A1B3, A3B2, and B2B3 were created. The rice straw weight losses of A1A3, A1B3, A3B2, and B2B3 were 43.24±6.73%, 32.18±1.19%, 44.01±2.93%, and 38.56±2.83%, respectively, which were significantly higher than those of A1 and B2, but lower than those of A3 and B3, respectively (Fig. 2B). On the third day, the pH of A1A3, A1B3, A3B2, and B2B3 was 6,6.5, 6.3, and 6.1, and increased to 7.1, 7.4, 8.2, and 7, on the 9th day, respectively (Fig. 2D). All returned to neutral and then became weakly alkaline. The combined microbial communities all followed the rule of pH fluctuating within 6-9 range, similar to other stable and efficient lignocellulose-decomposing microbial communities in previous reports (Liu et al., 2006). Acid-base combinations did not obtain a more efficient lignocellulose-decomposing microbial community, while the pH was more similar to a stable community.

Effect of inoculum source on the enrichment of microbial communities

Based on the T-test results, group A and group B's degradation ratio was not significantly different. However, the parallelity of group B was much higher than A. Therefore, both D1 and D2 had potential, while D2 was more suitable to screen high-efficiency microbial communities (Fig. 3). Bacterial biodiversity was higher in the thermophilic period than in the ripening period. The bacteria composition of both compost samples was significantly different, which resulted in group A and group B also exhibiting significant differences. The inoculum source had a greater impact on restrictive screen results. In this study, composting during the thermophilic period was a better choice of inoculum source than during the ripening period.

Composting in the thermophilic period is often used as an inoculum source to screen cellulose-degrading bacteria (Tesfaw and Assefa, 2014). For D2, the temperature of the compost body in the thermophilic period was higher, and organic matter decomposed rapidly. Cellulose materials decomposed more rapidly during this period, and bacteria with cellulose degradation abilities were also the most active. For D1, in the ripening period, the rapid decomposition of the organic matter ended.

The structure of the abtained microbial communities after restrictive screen culture

A significant number of OTUs in D1 and D2 were eliminated (Fig. 4A and B). The number of retained OTUs in A1, A2, and A3 were 337, 310, and 418, respectively, with B1, B2, and B3 retaining 408, 392, and 353, respectively. In addition, some OTUs that were not detected in D1 and D2 were detected in our microbial communities. A1, A2, and B2 had 827, 807, and 713 OTUs that were not found in D1, while B1, B2, and B3 had 1128, 862, and 663 OTUs that were not identified in D2.

The retained bacteria (amount ratio more than 0.1%) are shown in Table 1. Bacteria such as *Cellulosilyticum* have the ability to degrade cellulose. In the community, EMSD5, *Cellulosilyticum*, and other bacteria, and fungi synergistically degrade whole corn with cobs removed (Zhu et al., 2016). In the community SV79, which decomposes various lignocellulosic substrates to produce ethanol, *Cellulosilyticum* and *Acetivibrio, Clostridium, Ruminococcus*, and *Sporomusa* are the dominant bacteria (Zhao et al., 2014). *Treponema primitia* in the intestine of termites can degrade lignin (Lucey and Leadbetter, 2014). *Pseudomonas, Comamonas*, and *Lachnoclostridium* can also decompose cellulose in a composite community (Liu et al., 2011; Xue et al., 2020; Zagrodnik et al., 2021).

Table 1
The bacteria microbiome covered all samples(quantitative proportion above 0.1%)

Taxonomy	Quantitative proportion (%)											
	A1	A2	A3	B1	B2	B3	D1	D2	A1A3	A1B3	A3B2	B2B3
Alcaligenes	67.1	59.0	10.7	2.2	3.4	1.4	1.7	1.1	2.6	6.1	6.8	4.2
Lachnoclostridium	6.0	0.8	1.4	0.8	1.2	4.1	1.0	0.6	0.5	0.3	0.8	1.8
Cellulosilyticum	3.6	2.5	41.1	1.2	0.6	2.6	0.3	0.2	0.3	0.3	0.3	0.6
Proteiniphilum	3.6	5.1	20.4	2.4	0.7	2.1	0.3	0.2	4.9	3.5	0.7	2.5
unidentified	3.0	2.0	1.0	2.1	5.0	1.0	0.2	1.0	0.4	0.4	1.9	0.6
Comamonas	0.7	0.7	2.1	2.3	31.8	36.5	1.0	1.2	51.3	18.3	4.1	27.0
Pseudomonas	0.7	3.2	2.8	12.4	11.7	11.6	0.5	0.7	10.5	11.5	0.8	2.4
Advenella	0.6	0.3	0.5	47.8	4.1	1.7	0.3	0.2	0.6	3.1	4.4	6.3
Treponema	0.2	0.3	0.5	0.7	4.6	0.8	0.3	0.3	0.9	29.9	18.8	27.8
Clostridium	0.1	0.2	0.6	0.2	0.1	0.3	2.6	0.7	0.1	0.5	0.8	0.7

Some retained bacteria could not use cellulose directly, however, they can exist in communities that decomposed lignocellulose, such as *Alcaligenes.* They can utilize organic acids and amino acids to produce ammonia and carbon dioxide. *Alcaligenes faecalis* have been reported in plants, soil, and other environments (Rosenberg, 2014), and *Alcaligenes sp* TB is an aerobic denitrifying bacterium (Chen et al., 2016). *Proteiniphilum* is widely reported in anaerobic fermentation sludge, as it cannot decompose lignocellulose and cellobiose (Maspolim et al., 2015). It can, however, use peptone, arabinose, etc., and its metabolites include small molecular organic acids, CO_2, and H_2 (Langer et al., 2016). *Treponema caldarium* is a strictly anaerobic hyperthermia spirochete, which uses glucose, lactose, and other sugars as substrates, and has been reported in a complex community that degraded naphthalene (Koelschbach et al., 2017). These bacteria may assist in the elimination of acidic byproduct that inhibits cellulose-degrade strains.

Some bacteria, like *Sporomusa*, *Proteiniclasticum*, Dethiosulfovibrionaceae, *Desulfotomaculum*, *Desulfosporosinus*, *Pseudoxanthomonas*, *Nocardia*, *Alteromonadaceae*, *Erysipelotrichaceae*, *Novosphingobium*, *Propionigenium*, *Nonomuraea*, *Balneimonas*, and *Arcobacter* which were found in all the six enriched communities, but not in D1 or D2. These newcomers may play specific roles in the microbial community and may have originated from the compost. Their abundance in compost may be extremely low and difficult to detect with high throughput sequencing. After the growth environment changed, their abundance amount increased, allowing for detection with high-throughput sequencing. Additionally, they may also come from the air as the PCS medium was not sterilized. Generally, the enriched microbial community needs to have a high anti-pollution capacity. It was not hortative to be operated in a sterile environment such as an ultra-clean workbench, and thus other bacteria may contaminate them (Himanshu et al., 2017).

The abundance and composition of fungal OTUs from our communities were similar, which were different from that of the compost (Fig. 4C). While many fungi were eliminated as a result of the restricted culture, some survived, though their OTUs were less abundant. Some may be involved in straw catabolism, such as *Penicillium* (Ogunyewo et al., 2020), while most fungi had little connection to straw decomposition, like *Guehomyces pullulans* (Nakagawa et al., 2006) and *Hyphopichia burtonii* (Groenewald and Smith, 2010).

Effect of acid-base combination

The change of microbial community structure by an acid-base combination

Among the obtained communities by acid-base combination, A1A3 had the lowest pH (6.0) on the 3rd day and had a greater degradation ratio (43.24±6.73%). In contrast, A1B2 had the highest pH (6.5), and the degradation ratio was only 32.18±1.19%. Therefore, analysis was focused on these two communities to elucidate the mechanism of acid-base combination.

A1A3 was combined within group A, and A1B3 was combined between group A and B. Before mixing, the dominant bacteria in A1 was *Alcaligenes*, with a ratio of 67% (Table 1, Fig. 5), which cannot decompose cellulose. *Alcaligenes* can use peptone and yeast extract powder in PCS medium and produce alkaline substances, inducing a pH increase. The relative abundance of *Cellulosilyticum* was less than 3.6%, other bacteria producing acid were much lower than *Alcaligenes*. The dominant bacteria of A3 was *Cellulosilyticum*, followed by *Proteiniphilum*, while the relative abundance of *Alcaligenes* was 11%. The dominant bacteria of B3 was *Commonas*, followed by *Pseudomonas*. The relative abundance of *Lachnoclostridium*, which may have the ability to decompose cellulose, was only 4%. The total proportion of *Advenella*, *Alcaligenes*, and *Bordetella*, which belong to Alkalobacillaceae, was 4.8%.

After the combination, the dominant bacterium of A1A3 was *Comamonas*, which was different from A1 and A3, and the proportion of *Hydrogenispora* increased to 12%. The database information may be *hydrogenase poraethanolica*, a strictly anaerobic bacteria producing hydrogen and ethanol (Liu et al., 2014). The relative abundance of Alcaligenes was 3%. After the combination, the dominant strain of A1B3 was *Treponema*, followed by *Comamonas*, *Pseudomonas*, *Anaerophaga*, and *Alcaligenes*.

Whether it was the combination within one group or between two groups, the dominant bacteria varied greatly. After combination, the ratio of *Cellulosilyticum* decreased, the *Pseudomonas* ratio increased, and *Clostridium* ratio was low. Those three genera all have the potential ability to decompose lignocellulose. Their total proportions in A1, A3, and B3 were 4.4%, 44.5%, and 14.5%, respectively. The relative abundance of them in A1A3 and A1B3 was 10.9%, 12.3%, respectively. The numerical relationship was A3>A1A3>A1, B3>A1B3>A1. A1A3 and A1B3 were significantly higher than A1 before the combination but lower than A3 and B3. The results were consistent with straw degradation. The pH of A1 was higher than 6.6 (Fig. 2A), and the proportion of *Alcaligenes* in A1 was the highest corresponding. After the combination, the proportion of *Alcaligenes* in A1A3 and A1B3 is lower than that in A1, and their pH decreased below 6.5.

Change of fungi in the process of acid-base combination

Generally, all obtained fungal communities spatially overlapped, indicating a similar species diversity (Fig. 4C). Take A3B2, for example; the proportion of dominant fungi (1%) in the communities is shown in Fig. 6. Before the combination, the dominant fungi in A1 were *Scedosporium, Candida, Machimura, Penicillium*, etc. The dominant fungi in B2 were *Candida, Machimura, Acremonium, Penicillium, Scedosporium, IsSatchenkia*. After combination, the dominant fungi in A3B2 included *Candida, Machimura, Scedosporium, Acremonium, Penicillium, and Clavulina*. For fungi, the acid-base combination was just a simple mixing process. The dominant fungi were gathered into the new community, while the proportions likely changed as a result of competition.

Discussion

The screened bacteria can be divided into three groups. The first group exhibited lignocellulose decomposing functions, such as *Clostridium*, *Cellulosilyticum, Pseudomonas*, etc. The second group was likely to have auxiliary functions, such as *Alcaligenes, Pseudomonas* (decomposing bacteria or auxiliary bacteria), etc. The third group was not directly related to the decomposition of lignocellulose by current reports. It may have unknown functions or may be eliminated in the subculture in the future.

While in the screening process, the randomness was apparent. The obtained microbial communities were significantly different with the same inoculum source and under the same culture conditions. For example, the community structure of A1 was significantly different from that of parallel A2 and A3, and the straw degradation rate was much lower than that of A2 and A3.

The acid-base combination aimed to obtain a microbial community with a stable and robust ability to decompose straw. The goal is to optimize the variety of different microbiota-producing acids and alkaline without damaging the synergistic relationship between microorganisms in nature. The pH of the obtained microbiota decreased rapidly with the decomposition of straw before returning to a slightly alkaline level.

The regular change of pH was closely related to the structure of the community. The composition of dominant bacteria varied greatly, whether within or between groups. The cellulose-degrading strains represented by *Clostridium* can metabolize straw to produce small molecular organic acids, reducing the pH of the solution. Helper strains represented by *Alkalobacillus* can metabolize organic acids and amino acids to produce ammonia and carbon dioxide, increasing the solution's pH. When the auxiliary bacteria were dominant in the microbiota, their abundance was very high, such as A1, while the abundance of other strains was low. The decomposition ability of microbial bacteria was insufficient, and the pH was difficult to decline. When the number of auxiliary bacteria in the community was low, such as A3, B3, pH only slowly rose back to slightly alkaline. After the acid-base combination, the microbial community was reconstituted. The degrading and auxiliary strains were adjusted to a more appropriate quantity and proportion, and unrelated strains were knocked out simultaneously, which changed the pH regularly. The acid-base combination was conducive to obtaining an efficient and stable microbial community and correcting microbial communities' reduced decomposition ability caused by a random process in the restricted culture process.

Composting in the thermophilic period was a better choice of inoculum source than that of the ripening period. Many bacteria and most fungi were eliminated after the restrictive screening. Degrading strains, such as *Clostridium* and *Pseudomonas*, synergistic microbial such as *Pseudoxanomonas* and *Alkalobacillaceae* were obtained. Microbial communities were re-constituted by acid-base combination. The degrading and synergistic strains were adjusted to a more appropriate proportion, which changed the pH regularly. Acid-base combination fixed the instability of microbial communities caused by randomness of restrictive screening enrichment. The combination of the two techniques is high conducive to obtain efficient lignocellulose degradation microbial resources.

Declarations

Acknowledgements

This research was financially supported by the National Natural Science Foundation of China (NO. 31900107) .

Authors’ Contributions

Binbin Hua: Investigation, Data curation, Formal analysis, Validation, Writing - original draft, Writing - review & editing. Xiaofen Wang: Conceptualization, Methodology, Supervision, Writing-review & editing. Zongjun Cui: Supervision,Conceptualization.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding Information

This research was financially supported by the National Natural Science Foundation of China (NO. 31900107) .

References

1. Bartram AK, Lynch MDJ, Stearns JC, Moreno-Hagelsieb G, Neufeld JD (2011) Generation of Multimillion-Sequence 16S rRNA Gene Libraries from Complex Microbial Communities by Assembling Paired-End Illumina Reads. Appl Environ Microb 77(15):5569–5569**DOI**
2. Chen J, Gu S, Hao H, Chen J (2016) Characteristics and metabolic pathway of Alcaligenes sp. TB for simultaneous heterotrophic nitrification-aerobic denitrification. Appl Microbiol Biot 100(22):9787–9794**DOI**
3. Cui Z, Li M, Piao Z, Huang Z, Ishii M, Igarashi Y (2002) Selection of a composite microbial system MC1 with efcient and stability cellulose degradation bacteria and its function. Environ Sci 23:36–39**DOI**
4. Estrela S, Sánchez Á, Rebolleda-Gómez M (2021) Multi-Replicated Enrichment Communities as a Model System in Microbial Ecology. Front Microbiol 12:657467**DOI**
5. Groenewald M, Smith MT (2010) Re-examination of strains formerly assigned to Hyphopichia burtonii, the phylogeny of the genus Hyphopichia, and the description of Hyphopichia pseudoburtonii sp. nov. Int J Syst Evol Micr 60(11):2675–2680**DOI**
6. Guo P, Zhu W, Wang H, Lu Y, Wang X, Zheng D, Cui Z (2010) Functional characteristics and diversity of a novel lignocelluloses degrading composite microbial system with high xylanase activity. J Microbiol Biotechnol 20(2):254–264**DOI**
7. Himanshu H, Voelklein MA, Murphy JD, Grant J, O'Kiely P (2017) Factors controlling headspace pressure in a manual manometric BMP method can be used to produce a methane output comparable to AMPTS. Bioresour Technol 238:633–642
8. Hua B, Dai J, Liu B, Zhang H, Yuan X, Wang X, Cui Z (2016) Pretreatment of non-sterile, rotted silage maize straw by the microbial community MC1 increases biogas production. Bioresour Technol 216:699–705
9. Hua B, Lu Y, Wang J, Wen B, Cao Y, Wang X, Cui Z (2014) Dynamic Changes in the Composite Microbial System MC1 During and Following its Rapid Degradation of Lignocellulose. Appl Biochem Biotech 172(2):951–962**DOI**
10. Hui W, Jiajia L, Yucai L, Peng G, Xiaofen W, Kazuhiro M, Zongjun C (2013) Bioconversion of un-pretreated lignocellulosic materials by a microbial consortium XDC-2. Bioresour Technol 136:481–487**DOI**
11. Jawed K, Yazdani SS, Koffas MA (2019) Advances in the development and application of microbial consortia for metabolic engineering.Metabolic Engineering Communications,9. https://doi.org/10.1016/j.mec.2019.e00095
12. Kato S, Haruta S, Cui ZJ, Ishii M, Igarashi Y (2004) Effective cellulose degradation by a mixed-culture system composed of a cellulolytic Clostridium and aerobic non-cellulolytic bacteria. FEMS Microbiol Ecol 51(1):133–142

13. Koelschbach JS, Mouttaki H, Pickl C, Heipieper HJ, Meckenstock RU (2017) Rectinema cohabitans gen. nov., sp. nov., a rod-shaped spirochete isolated from an anaerobic naphthalene-degrading enrichment culture. Int J Syst Evol Microbiol 67(5):1288–1295**DOI**
14. Langer T, Hahnke S, Klocke M, Koeck DE (2016) Description of Proteiniphilum saccharofermentans sp. nov., Petrimonas mucosa sp. nov. and Fermentimonas caenicola gen. nov., sp. nov. isolated from mesophilic lab-scale biogas reactors and emended description of the genus Proteiniphilum.Int. J. Syst. Evol. Microbiol., 66(3). **DOI**: 10.1099/ijsem.0.000902
15. Liu J, Wan L, XiuHong X, HongTao L (2011) Effect of Cellulose-decomposing Strain on Microbial Community of Cow Manure Compost. Environ Sci 32(10):3073**DOI**
16. Liu JB, Wang WD, Yang HY, Wang XF, Gao LJ, Cui ZJ (2006) Process of rice straw degradation and dynamic trend of pH by the microbial community MC1. J Environ Sci (China) 18(6):1142–6**DOI**
17. Liu Y, Qiao JT, Yuan XZ, Guo RB, Qiu YL (2014) Hydrogenispora ethanolica gen. nov., sp nov., an anaerobic carbohydrate-fermenting bacterium from anaerobic sludge. Int J Syst Evol Micr 64(5):1756–1762**DOI**
18. Lucey KS, Leadbetter JR (2014) Catechol 2,3-dioxygenase and other meta-cleavage catabolic pathway genes in the 'anaerobic' termite gut spirochete Treponema primitia. Mol Ecol 23(6):1531–1543**DOI**
19. Maspolim Y, Zhou Y, Guo C, Xiao K, Ng WJ (2015) The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation.Bioresource Technol. **DOI**: 10.1016/j.biortech.2015.04.087
20. Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8(19):4321–5**DOI**
21. Nakagawa T, Ikehata R, Uchino M, Miyaji T, Takano K, Tomizuka N (2006) Cold-active acid beta-galactosidase activity of isolated psychrophilic-basidiomycetous yeast Guehomyces pullulans. Microbiol Res 161(1):75–79**DOI**
22. Ogunyewo OA, Randhawa A, Gupta M, Kaladhar VC, Yazdani SS (2020) Synergistic Action of a Lytic Polysaccharide Monooxygenase and a Cellobiohydrolase from Penicillium funiculosum in Cellulose Saccharification Under High Substrate Loading.Appl. Environ. Microb., 86(23). **DOI**: 10.1128/AEM.01769-20
23. Rosenberg E, Delong EF, Lory S (2014) et al The Prokaryotes:The Family Methylobacteriaceae[J] Chapter 256313–340. 10.1007/978-3-642-30197-1
24. **S., H., Z., C., Z., H., M., L., M., I., Y., I., Construction of a stable microbial community with high cellulose-degradation ability**.Appl. Microbiol. Biot., **59**(**4-5**),**529-534**. **DOI**: 10.1007/s00253-002-1026-4
25. Tesfaw A, Assefa F (2014) Co-culture: A great promising method in single cell protein production. Biotechnology and Molecular Biology Reviews 9(2):12–20
26. Wang J, Zhu D, Zhao S, Xu S, Yang R, Zhao W, Zhang X, Huang Z (2021) Effect of liquid volume and microflora source on degradation rate and microbial community in corn stover degradation.AMB Express, 11(1). **DOI**: 10.1186/s13568-021-01233-5
27. Wang R, Zhao S, Wang Z, Koffas MA (2020) Recent advances in modular co-culture engineering for synthesis of natural products. Curr Opin Biotech 62:65–71
28. Wang W, Yan L, Cui Z, Gao Y, Wang Y, Jing R (2011) Characterization of a microbial consortium capable of degrading lignocellulose. Bioresour Technol 102(19):9321–9324
29. Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biot 85(4):849–860**DOI**
30. Wen B, Yuan X, Li QX, Liu J, Ren J, Wang X, Cui Z (2015) Comparison and evaluation of concurrent saccharification and anaerobic digestion of Napier grass after pretreatment by three microbial consortia. Bioresour Technol 175:102–111**DOI**

31. Xu C, Yu H (2021) Insights into constructing a stable and efficient microbial consortium. Chinese J Chem Eng 30:112–120
32. Xue C, Zhang Q, Owens G, Chen Z (2020) A cellulose degrading bacterial strain used to modify rice straw can enhance Cu(II) removal from aqueous solution. Chemosphere 256:127142**DOI**
33. Yu J, Zhao Y, Zhang H, Hua B, Yuan X, Zhu W, Wang X, Cui Z (2017) Hydrolysis and acidification of agricultural waste in a non-airtight system: Effect of solid content, temperature, and mixing mode. Waste Manage 59:487–497**DOI**
34. Yuan X, Cao Y, Li J, Wen B, Zhu W, Wang X, Cui Z (2012) Effect of pretreatment by a microbial consortium on methane production of waste paper and cardboard. Bioresour Technol 118:281–288**DOI**
35. Yuan X, Ma L, Wen B, Zhou D, Kuang M, Yang W, Cui Z (2016) Enhancing anaerobic digestion of cotton stalk by pretreatment with a microbial consortium (MC1). Bioresour Technol 207:293–301**DOI**
36. Zagrodnik R, Duber A, Seifert K (2021) Hydrogen production during direct cellulose fermentation by mixed bacterial culture: The relationship between the key process parameters using response surface methodology. J Clean Prod 314:127971**DOI**
37. Zhao C, Deng Y, Wang X, Li Q, Huang Y, Liu B (2014) Identification and characterization of an anaerobic ethanol-producing cellulolytic bacterial consortium from Great Basin hot springs with agricultural residues and energy crops. J Microbiol Biotechnol 24(9):1280–1290**DOI**
38. Zhu N, Yang J, Ji L, Liu J, Yang Y, Yuan H (2016) Metagenomic and metaproteomic analyses of a corn stover-adapted microbial consortium EMSD5 reveal its taxonomic and enzymatic basis for degrading lignocellulose. Biotechnol Biofuels 9(1):243**DOI**

Figures

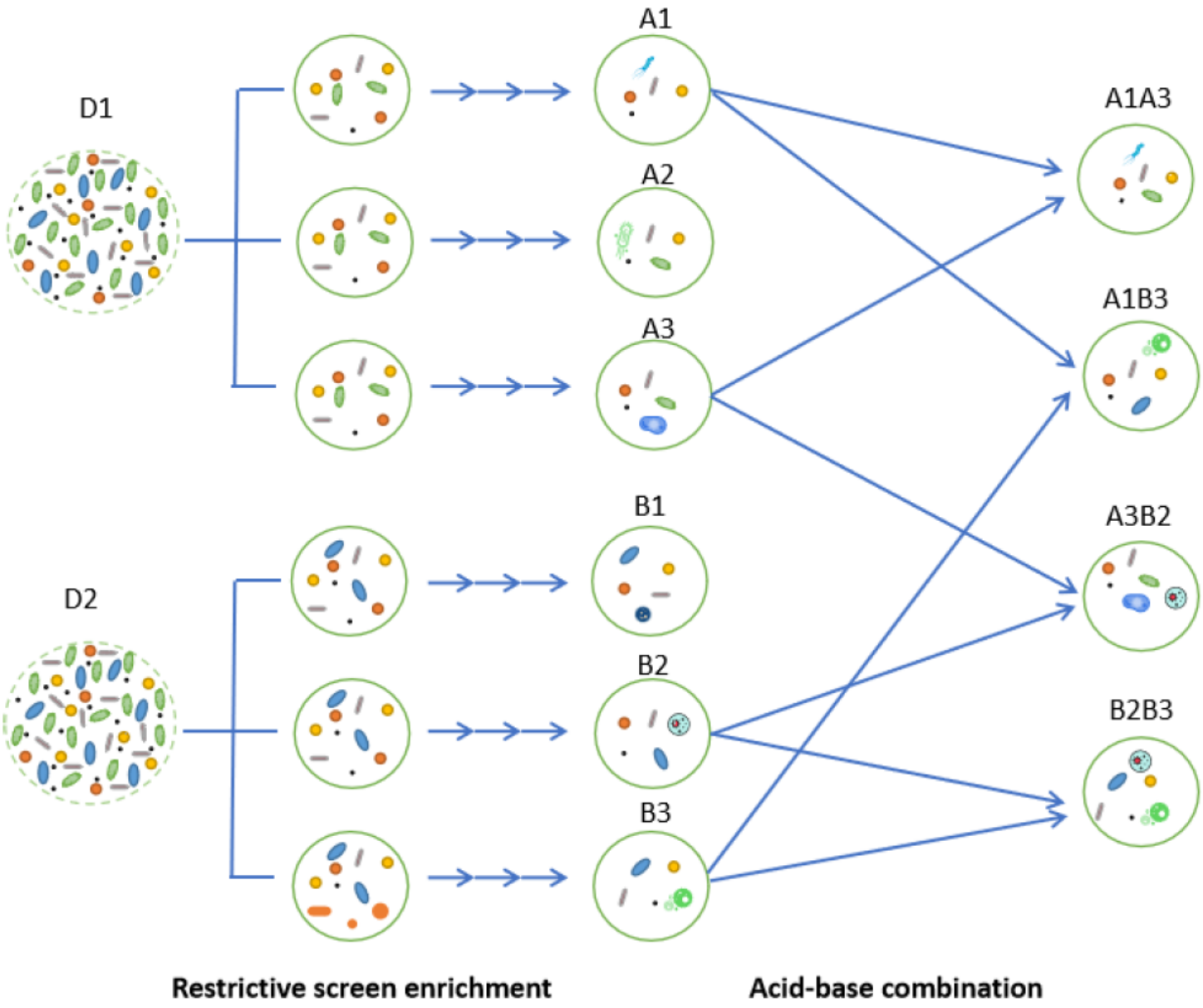

Figure 1

Schematic diagram of experimental design process

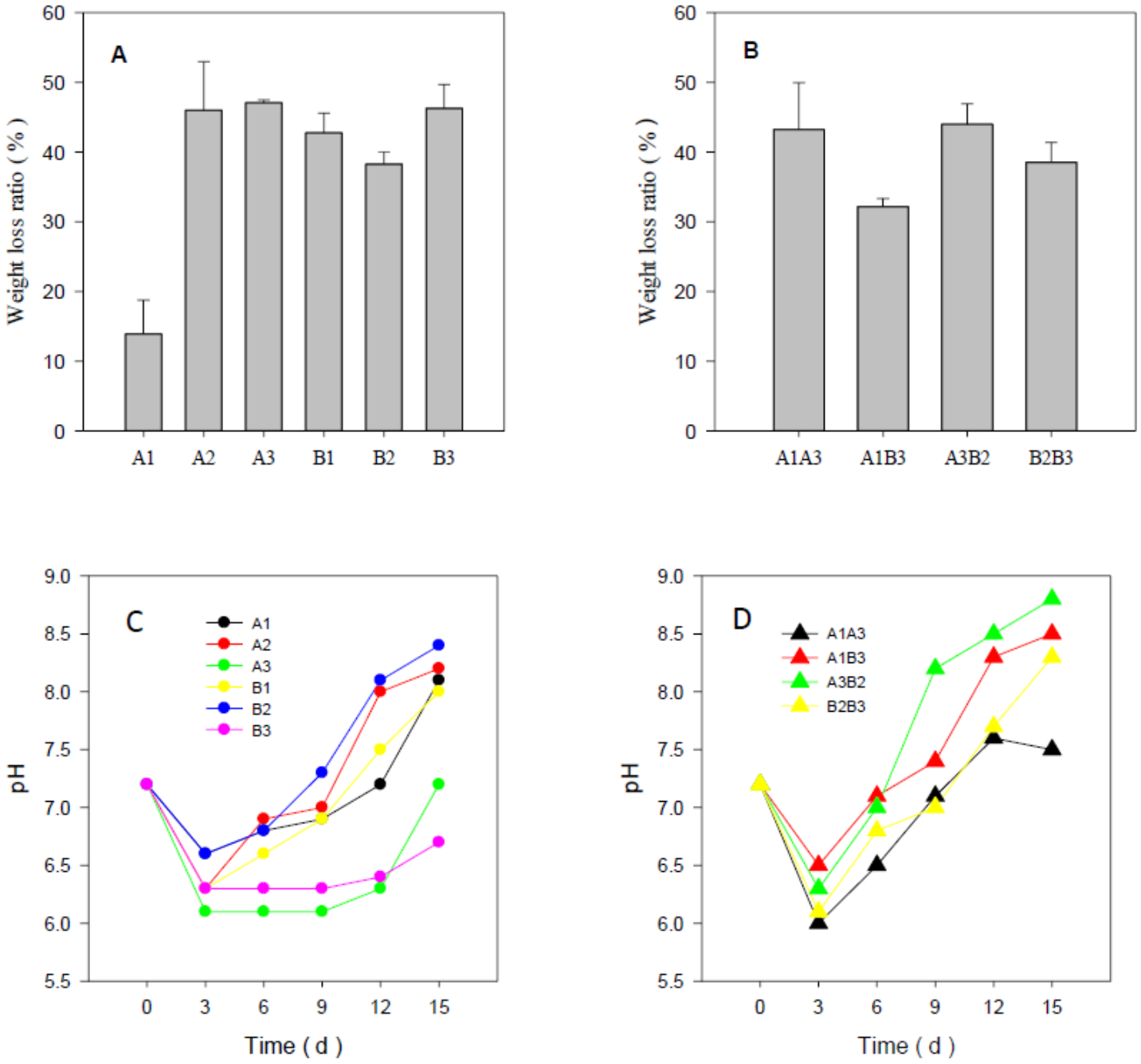

Figure 2

The weight loss ratio of rice straw dynamic change of pH in microbial communities. A represents six directly enriched microbial communities and B represents four acid and alkaline mixed microbial communities. C represents six directly enriched microbial communities and D represents four acid and alkaline mixed microbial communities.

Figure 3

Bacteria community structure of each group (genus level). “g” represents genus, after “_” are genus names. Other means reference sequence is not found in the database.

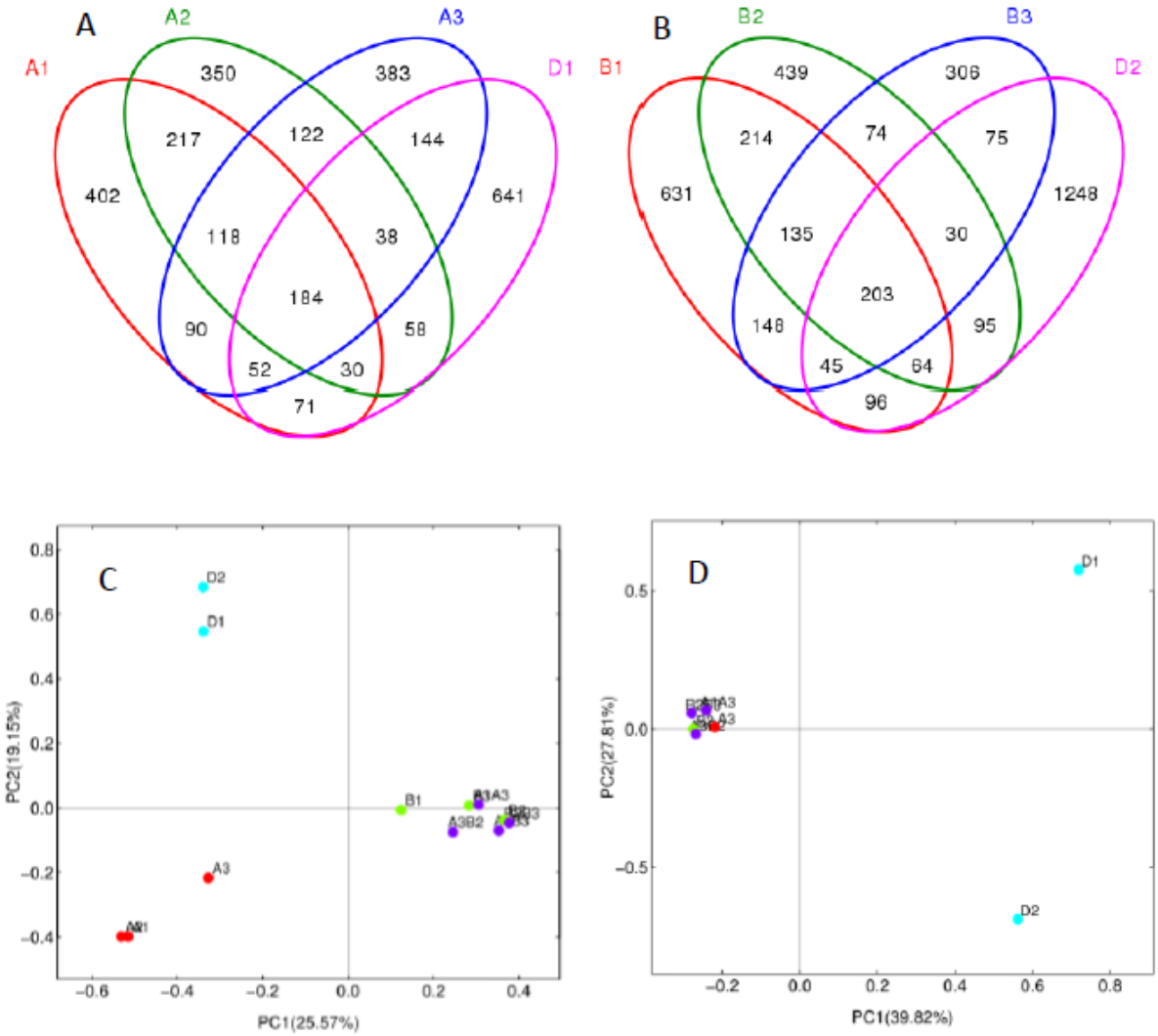

Figure 4

OTUs venn analysis of microbial community A1, A2, A3 and D1(A); and OTUs venn analysis of microbial community B1, B2, B3 and D2(B); PCA analysis of community diversity based on OTU abundant; (C) is the PCA analysis of bacteria , and (D) is the PCA ananlysis of fungi.

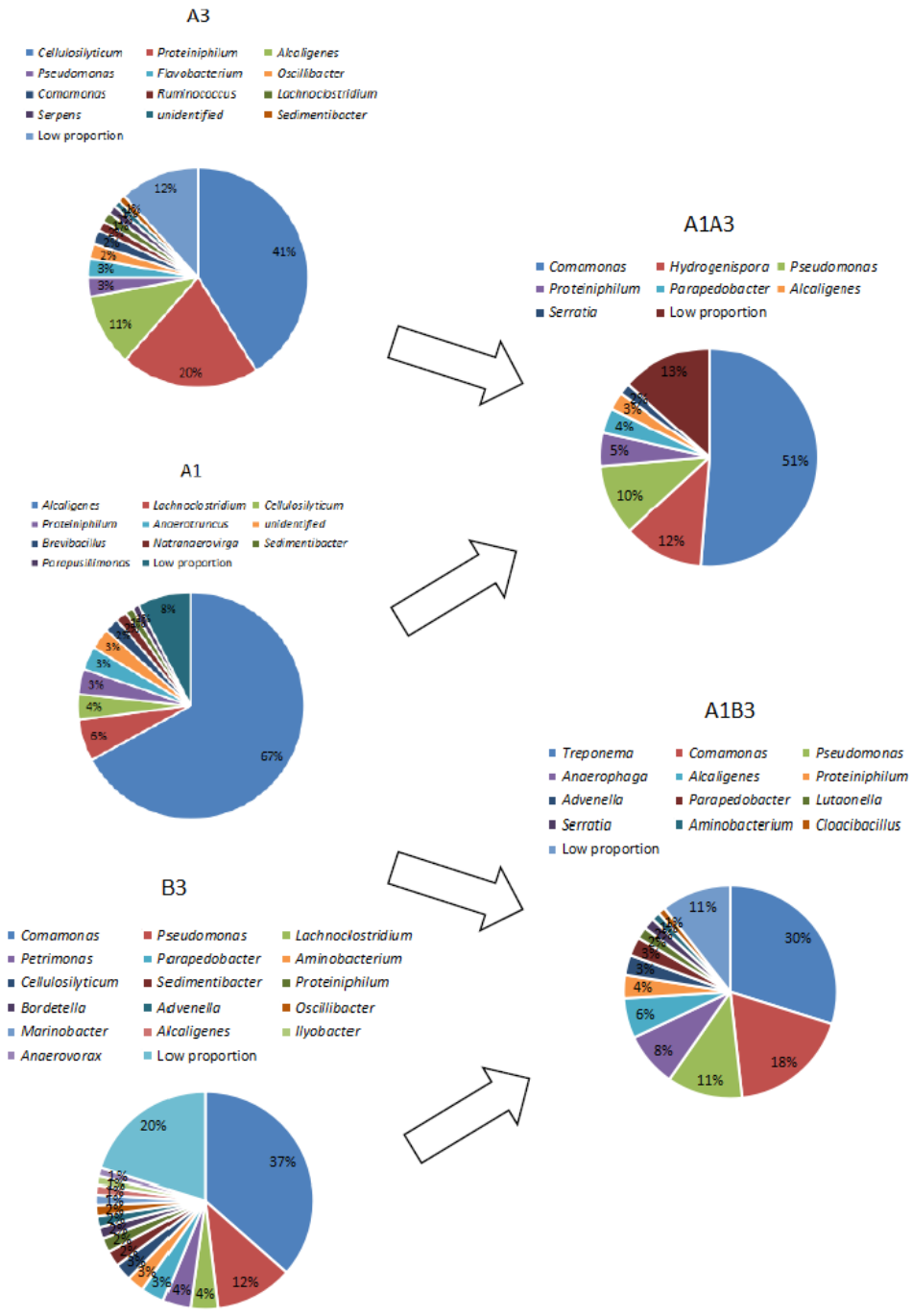

Figure 5

The dominant bacteria in microbial community A1, A3, B3, A1A3 and A1B3 (genus level).The quantitative proportion lower than 1% are combined as “low proportion”

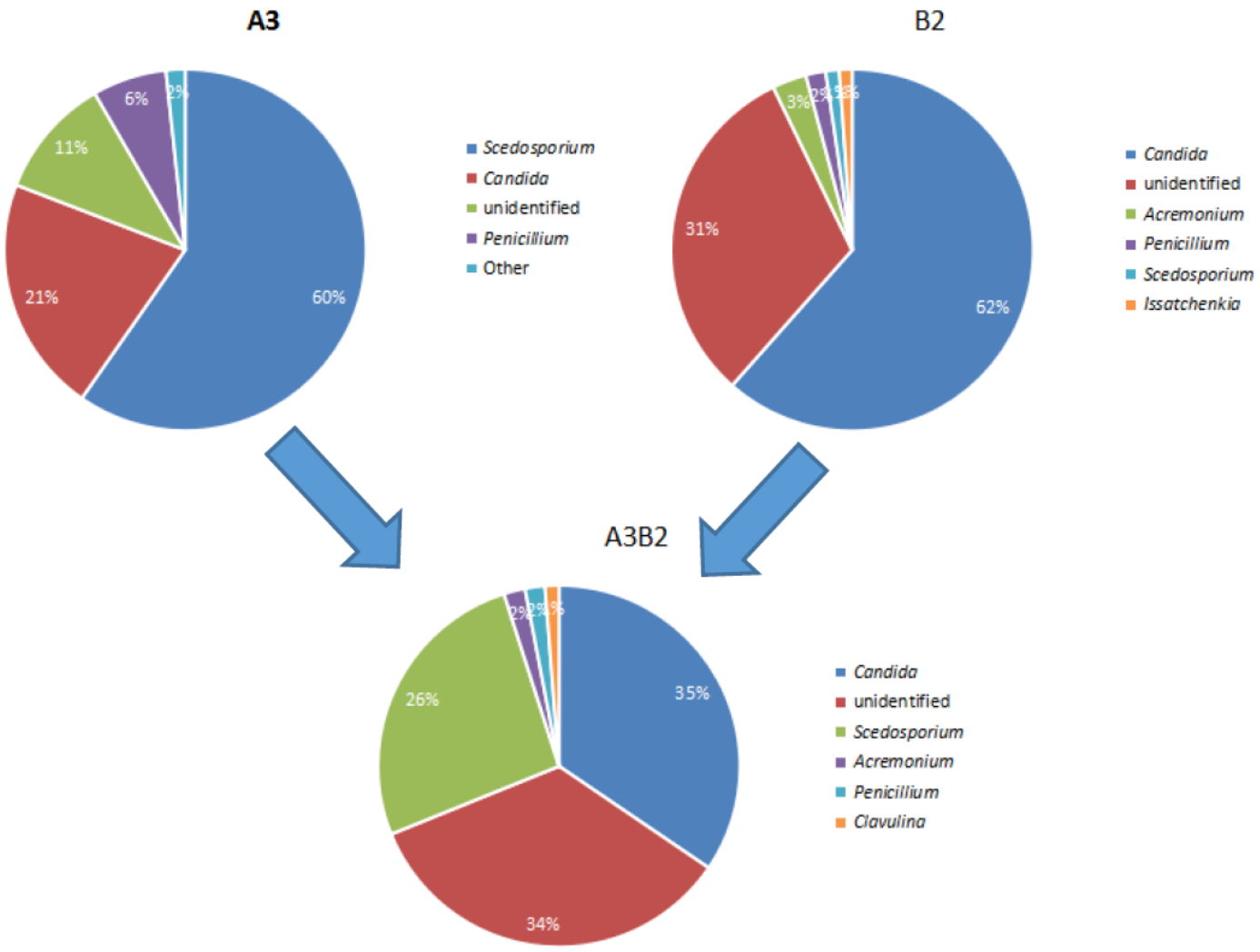

Figure 6

The preponderant fungus (quantitative proportion above 1%, genus level) in microbial community A3, B2 and A3B2. Unidentified means lack of identified genus level name in the database.Other means no reference sequnance in the database.