

The metabolic mechanism of growth inhibition by co-culture of *Bacteroides xylanisolvens* Y-11 and *Bifidobacterium longum* y37

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Abstract

Bacteroides xylanisolvens Y-11 and *Bifidobacterium longum* y37 isolated from human gut were found to inhibit each other's growth after co-culturing in previous studies. To further reveal the potential mechanism of mutual inhibition between them, ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to investigate the metabolic changes of the strains after monoculture and co-culture, and the key differential metabolites were subject to the validation. The results showed that the types and amounts of metabolites were significantly changed during co-culture, with hydrocarbons and their derivatives, organic acids and esters being the main differential metabolites, which posed a greater influence on the metabolism of *B. xylanisolvens* Y-11 than on *B. longum* y37. Further studies suggest that cycloserine and succinic acid may be the main metabolites that inhibit the growth of both strains, and the decrease of pH may be the main reason for succinic acid to inhibit the growth of the two strains. Moreover, *B. longum* y37 played a dominant role in the co-culture and its metabolites influenced the growth of *B. xylanisolvens* Y-11 to a greater extent. This study provides a new perspective for further understanding of the interaction between intestinal microbes and the influence of intestinal microecology on the occurrence and development of diseases.

Keywords: co-culture, *Bacteroides xylanisolvens*, *Bifidobacterium longum*, metabolism.

Introduction

Co-culture of microorganisms is a method of culturing two or more strains based on known living environment and culture conditions. (Khan et al., 2018). Pure *cultures in vitro* may differ significantly from the way microorganisms grow in nature and within organisms (Kämpfer et al., 2017), where each microorganism in the natural environment can interact with other microorganisms and compete for space and nutrients. Compared with pure culture, co-culture not only simulates a more realistic environment (Goers et al., 2014) to investigate the interaction between multiple microorganisms (Smid and Lacroix, 2012), but also induces the production of bioactive secondary metabolites, obtains some unique products or increases the yield of products, etc. (Vinale et al., 2017, Bertrand et al., 2014), which is a better way to further understand microbial interactions. When the mutually antagonistic *Brevibacillus laterosporus* BL-21 was co-cultured with *Bacillus subtilis* HNDF2, it was found that the co-cultures showed better anti-phytopathogenic fungal activity than the pure cultures of both strains, and the weaker HNDF2 is able to stimulate the dominant bacterium BL-21 to produce higher concentrations of antimicrobial substances or even new ones (Xu et al., 2015). All of this indicates that co-culture not only increases the existing metabolites but also promotes the production of new metabolites, which is of great research value.

The intestine is an essential digestive and immune organ of the human body (Ochoa-Repáraz and Kasper, 2016). Not only playing a role in maintaining intestinal microecological homeostasis (Hao et al., 2019, Yoo et al., 2020), the gut microbiota has been increasingly found to be closely associated with numerous diseases such as diabetes, hypertension, and Alzheimer's disease in recent years (Kataoka, 2016, Zhang et al., 2021). Rather than sin-

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gle bacteria, intestinal microorganisms are composed of various bacteria that rely on synergy, mutual support, and biased symbiosis to maintain the gut in a stable state (Lagier et al., 2018). Meanwhile, the mixture of bacteria applied to the human micro-ecological environment has a greater buffering capacity and environmental adaptability. Moreover, the mixture of multiple intestinal microorganisms is in closer proximity to the real gut flora, which facilitates the in-depth study of the microecological mechanism of co-culture. But there are too many influence factors in this way, and a two-by-two mixture is more conducive to the study of gut microbial interactions. However, the intestinal tract is mostly anaerobic bacteria (Liu et al., 2021), which brings more challenges to co-culture.

Bifidobacterium longum is the most abundant *Bifidobacterium* in the human intestine (Rinninella et al., 2019), especially in the feces of infants, accounting for about 69.96% (LEE et al., 2015). It has been reported to have a wide range of probiotic functions, including allergy inhibition (Xiao et al., 2006), cholesterol reduction (Andrade and Borges, 2009, Jiang et al., 2021, Bordoni et al., 2013), improvement of skin health (Wong et al., 2019) and human health (Takahashi et al., 2006, Akatsu et al., 2012), and prevention of intestinal diseases (Akatsu et al., 2012, Yao et al., 2021). The sugar metabolism in *Bifidobacterium* spp. produces large amounts of organic acids such as acetic acid and lactic acid (Rodes et al., 2021). It can be added to functional dairy products as a co-culture being one of the most widely used probiotics. It has been reported that *Bacteroides* spp. constitute the major genus of the intestinal bacterial group and are involved in mutual antagonism of intestinal microorganisms, as well as in exploitative competition for nutrients (ZAFAR et al., 2021). *Bacteroides Xylanomycetes* from the human intestine produce succinate as the main end product through sugar fermentation (Chassard et al., 2008). It is considered to be one of the most potent gut bacteria promoted by hetero-terpene derivatives of *Ganoderma lucidum* (Qiao et al., 2020), has been reported to increase liver and blood folate content against fatty liver (Qiao et al., 2020), degrade nicotine (Bo et al., 2022), and have protective effects against smoking-related fatty liver disease. Meanwhile, *Bacteroides xylanisolvens* is safe for *in vivo* usage and its fermented dairy products have already been marketed as novel foods in the European Union. *Bifidobacterium longum* and *Bacteroides xylanisolvens* are both prevalent anaerobic genera in the gut, and no co-culture research on them has been reported.

In our previous study, we used macro-transcriptome sequencing to analyze feces from kidney stone patients and healthy humans, and constructed an association network of the top 50 dominant species in terms of abundance. Based on the microorganisms isolated from kidney stones and healthy human feces, we targeted *Bacteroides xylanisolvens* and *Bifidobacterium longum*, which were isolated as a pair of negatively associated regulatory species, for further validation. Therefore, *B. xylanisolvens* Y-11 and *B. longum* y37 were co-cultured *in vitro*, and the results showed that they did have a mutually inhibitory effect. To further elucidate their potential mechanisms,

this study was conducted to investigate the metabolism of the two strains in monoculture and co-culture using non-targeted metabolomic techniques, aiming to reveal the mechanism of intestinal microbial interaction and provide new ideas for the subsequent research on the roles of intestinal microbial in disease formation and disease prevention.

Materials and Methods

Activation of strains

The strains *Bacteroides xylanisolvens* Y-11 and *Bifidobacterium longum* y37 were isolated from human fecal samples. Both of them stored at 4°C were transferred to new anaerobic tubes containing GAM medium (pH 7.3±0.1) for activation and incubated at 37°C for 24 h to 36 h. The concentration of the bacterial liquid solution was adjusted to the same level ($OD_{600\text{ nm}} = 0.5$) and used for later experiments.

Inoculation of strains

A monoculture of strains was obtained by inoculating 1 mL each of *B. xylanisolvens* Y-11 and *B. longum* y37 at the same initial concentration into a new 20 mL of GAM liquid medium. Co-culture was obtained by inoculating the same medium in an equal proportion with the mono-culture of the two bacterial stock solutions.

Analysis of metabolites in fermentation broth

The prepared bacterial broth was incubated at 37°C for 48 h. Subsequently, the fermentation broth of *B. xylanisolvens* Y-11, *B. longum* y37 strain and co-culture strain were centrifuged at 10,000×g for 10 min at 4°C, and the supernatant was filtered with 0.45 µm aqueous filter head and loaded into the injection bottle, while 10 µL of each sample to be tested was made into quality control samples, and non-targeted metabolite positive and negative ions were detected by SCIEX Triple TOF 5600+, The parameters of liquid chromatographic conditions and mass spectrometry conditions refer to Tian (Tian et al., 2022), then analyzed by MS-DIAL, XCMS, MetaboAnalyst5.0 and KEGG database.

Validation of differential metabolites

Based on the results of the non-targeted metabolomic assay analysis, key differential metabolites were screened for validation to further reveal the mechanism. Eight substances that may affect the growth of the strain were initially identified: D-cycloserine, betaine, creatinine, L-threonine, proline, succinic acid, serine, and valine, which were added to 10 mL of basic GAM medium at different concentrations of 0.01 g/L, 0.03 g/L, and 0.05 g/L, respectively, and incubated at 37°C and recorded the changes in absorbance $OD_{600\text{ nm}}$ and pH at 0 h, 12 h, and 24 h, and compared to a control group without any added substance.

Statistical analysis of data

The experimental results were expressed as mean ± standard deviation, and one-way ANOVA and Tukey post hoc test were

used for multiple comparisons, and $P < 0.05$ was considered statistically significant.

Results

Chromatogram of UPLC-MS detection

The positive and negative total ion current (TIC) of quality control (QC) samples were shown in Figure 1. It could be seen that the curve overlap of total ion flow for metabolite detection was high and stable, which showed the signal stability of mass spectrometry was good for the same sample when detected at different times.

ing the complexity of the model and enhancing the explanatory power of the model, and the scores of each subgroup of OPLS-DA were plotted (Figure 3).

Volcano Plot Analysis of metabolites

A fast view of the differences in metabolite levels in the two groups and the statistical significance of the differences was obtained by Volcano Plot, with red indicating significantly upregulated metabolites, green indicating significantly down-regulated metabolites, and gray indicating non-significant metabolites. As can be seen in Figure 4, *B. xyloisolvans* Y-11 had

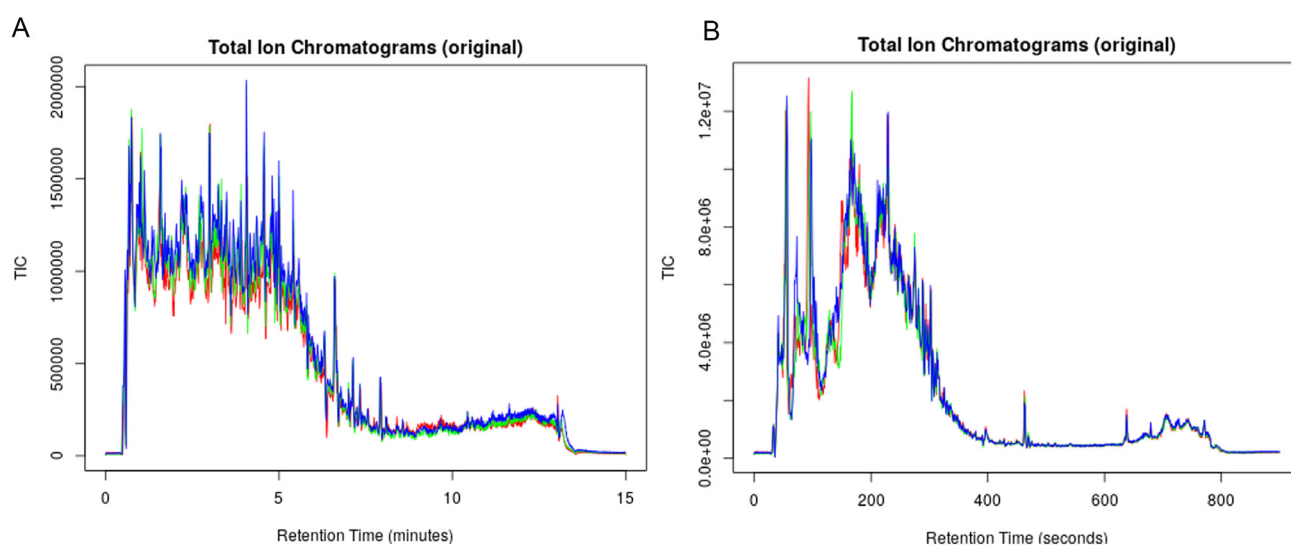


Figure 1. Total ion flow diagram (A. Negative ions, B. Positive ions)

Principal component analysis of metabolites

The principal component analysis (PCA) scores were plotted as follows (Figure 2), and the PCA figure revealed that the three groups of samples, as well as the QC, showed significantly different clustering characteristics, with better differentiation in the negative ions mode, where the samples within the group were well clustered, and the metabolite characteristics of *B. longum* y37 and co-cultures in the positive ions mode were partially overlapping, and overall, the distribution of microbial metabolites was significantly different between several groups of samples.

Discriminant analysis of metabolites by orthogonal partial least squares

As the PCA was insensitive to less correlated variables, for maximizing the distinction between groups and facilitating the search of differential metabolites, orthogonal partial least squares discriminant analysis (OPLS-DA) was used, to remove information irrelevant to classification better, effectively reduc-

more differential metabolites before and after co-culture, and far more differential metabolites were detected in the positive ions mode than in the negative ions mode. *B. longum* y37 detected more differential metabolites in the negative mode than in the positive ions mode when incubated alone. *B. xyloisolvans* Y-11 and *B. longum* y37 detected more differential metabolites in the positive ions mode than in the negative ions mode when co-cultured. More differential metabolites were detected in the positive ions mode than in the negative ions mode when co-cultured.

Screening, classification and identification of differential metabolites

A total of 4541 compounds were identified from the fermentation broth of the two strains, including 1560 hydrocarbons and their derivatives, 1369 organic acids and esters, 591 organic bases, 291 salts, 246 amino acids and their derivatives, 242 nucleotides and their derivatives, 105 heterocyclic compounds, 21 sugars, and 116 other compounds. The metabolites of *B.*

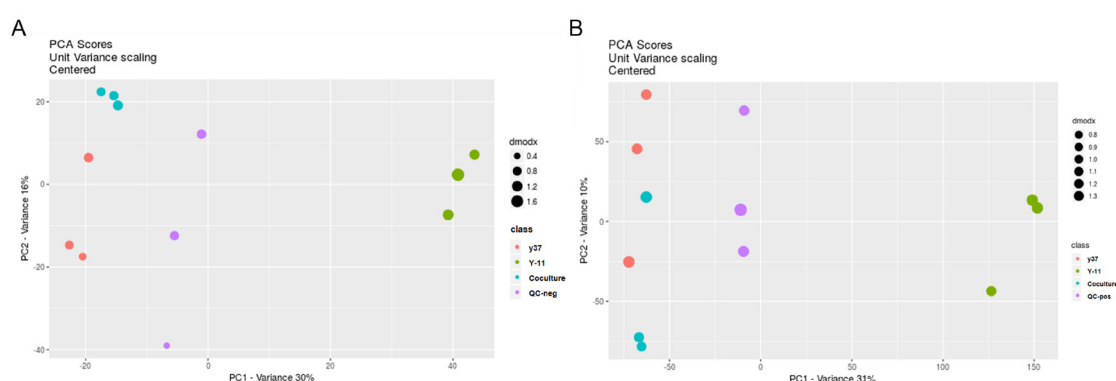


Figure 2. Principal component analysis graph (A. Negative ions, B. Positive ions). PC1 indicate the first principal component, PC2 indicate the second principal component, and the percentage indicate the explanation rate of this principal component for the data set; each point in the graph indicate a sample, and samples of the same group are represented using the same color, and Class is the grouping.

xylanisolvans Y-11 were more different after co-culture, where the positive ions mode identified a higher number of differential metabolites down-regulated, and *B. longum* y37 identified more differential metabolites in the negative ions mode than in

tabolites were detected in negative ions and positive ions mode before and after the co-culture of *B. xylanisolvans* Y-11, respectively, and 43 and 21 significant differential metabolites were detected in negative ions and positive ions mode before and

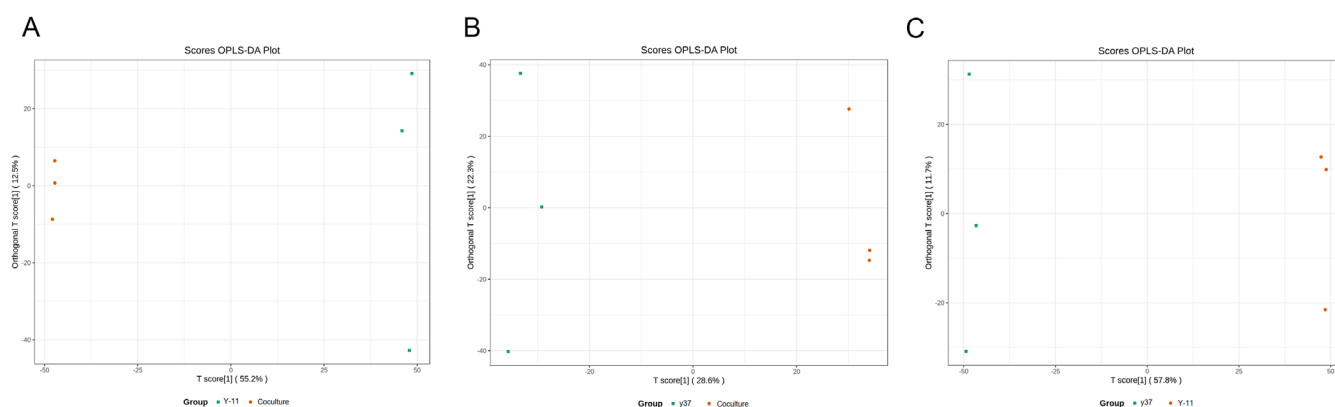


Figure 3. OPLS-DA score plots (A. Y-11 and co-culture, B. y37 and co-culture, C. y37 and Y-11). The horizontal coordinate indicates the predicted component score value, and the direction of the horizontal coordinate shows the gap between groups; the vertical coordinate indicates the orthogonal component score value, and the direction of the vertical coordinate shows the gap within groups; the percentage indicates the explanation rate of the component to the data set.

the positive ions mode. The differential metabolites matching $VIP \geq 1$, P value < 0.05 , fold change ≥ 2 and fold change ≤ 0.5 were selected together. 132 and 135 significant differential me-

after co-culture of *B. longum* y37, respectively (Table 1). The significantly different metabolites were detected in the negative ions and positive ions modes before and after co-culture for

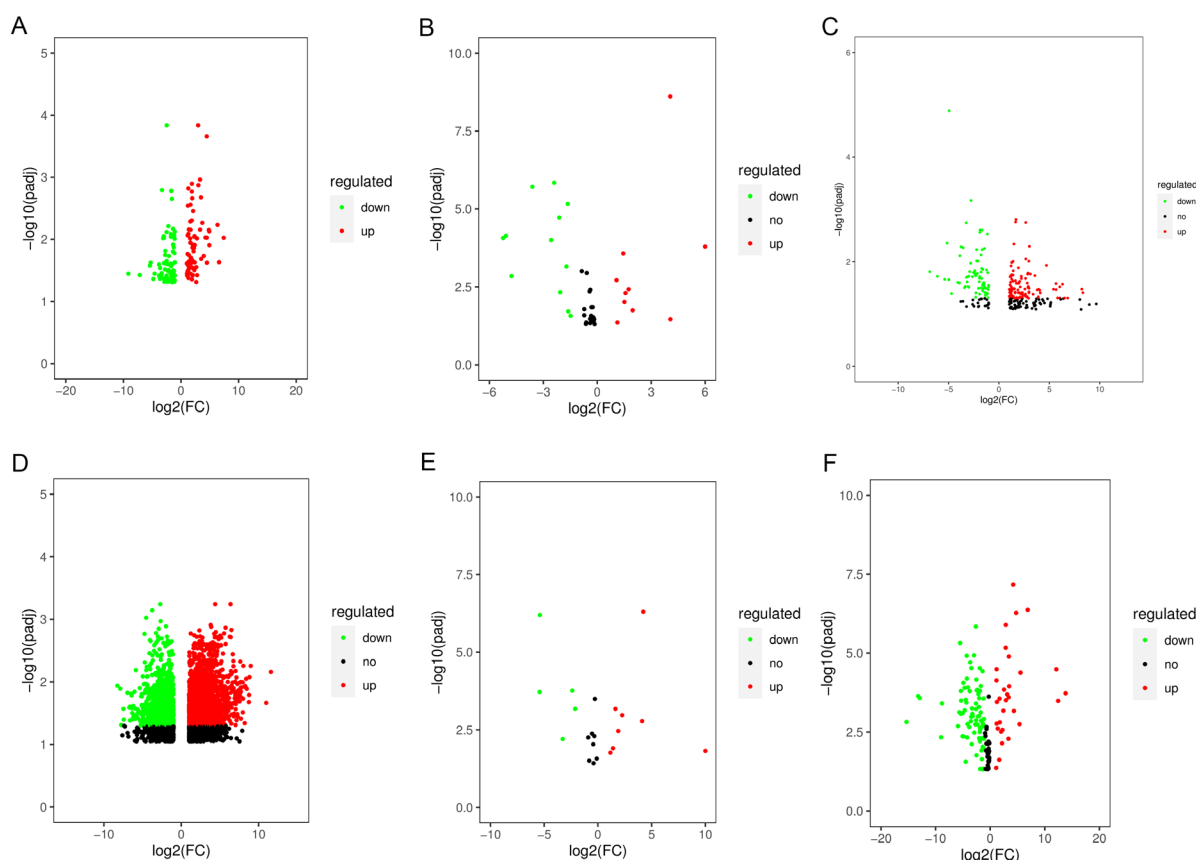


Figure 4. Volcano plot of differential metabolites (A. negative ion mode between Y-11 and co-culture, B. negative ion mode between y37 and co-culture, C. negative ion mode between y37 and Y-11; D. positive ion mode between Y-11 and co-culture, E. positive ion mode between y37 and co-culture, F. positive ion mode between y37 and Y-11). Each point in the volcano plot represents a metabolite, and the horizontal coordinate indicates the logarithmic value of the quantitative difference ploidy of a metabolite in two samples; the vertical coordinate indicates the VIP value. The larger the absolute value of the horizontal coordinate, the greater the difference in expression ploidy between the two samples; the larger the value of the vertical coordinate, the more significant the differential expression and the more reliable the differentially expressed metabolite obtained from the screening. The green dots in the graph represent downregulated differentially expressed metabolites, red dots represent upregulated differentially expressed metabolites, and black represents metabolites detected but not significantly different.

B. longum y37 (Table 1).

The statistics of significantly different metabolite categories (Table 2) showed that *B. xylanisolvens* Y-11 had the largest changes in organic acids and esters before and after co-culture, accounting for about 54.90%, of which 82 metabolites were down-regulated and 30 metabolites were up-regulated; followed by hydrocarbons and derivatives, accounting for about 17.16%, of which 19 metabolites were down-regulated and 16 metabolites were up-regulated. The largest changes in *B. longum* y37 before and after co-culture were organic acids and esters, accounting for about 35.71%, of which 12 metabolites were down-regulated and 8 metabolites were up-regulated; followed by hydrocarbons and derivatives, accounting for about 26.79%, of which 10 metabolites were down-regulated and 5 metabolites were up-regulated.

KEGG enrichment analysis of differential metabolites

KEGG pathway enrichment was performed based on differential metabolite results using metaboanalyst, MBRole 2.0, and the results are shown in Figure 5. The first three metabolic pathways enriched in *B. xylanisolvens* Y-11 and *B. longum* Y-37 before and after co-culture and in monoculture were different, among which enriched in *B. xylanisolvens* Y11 co-culture were Aminoacyl-tRNA biosynthesis, Purine metabolism Valine, leucine and isoleucine biosynthesis. While *B. longum* y37 was enriched to: Alanine, aspartate and glutamate metabolism, Pantothenate and CoA biosynthesis, Citrate cycle (TCA cycle). The two strains were enriched for Purine metabolism, D-Glutamine and D-glutamate metabolism, Aminoacyl-tRNA biosynthesis in monoculture. The KEGG pathways and the degree of enrichment of *B. xylanisolvens* Y-11 and *B. longum*

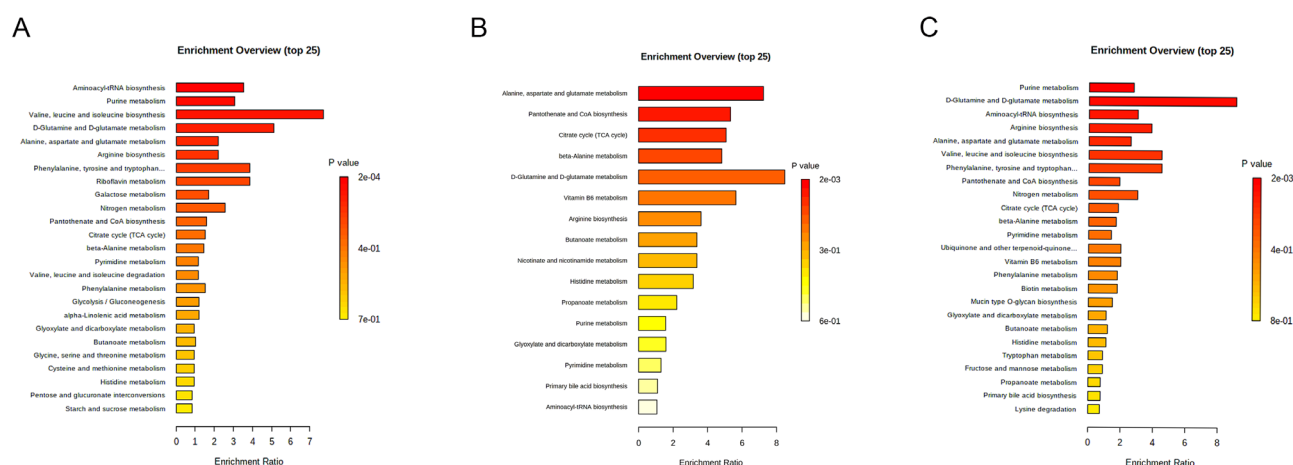


Figure 5. KEGG enrichment pathway diagram (A. Y-11 and co-culture, B. y37 and co-culture, C. y37 and Y-11). The vertical co-ordinate is the name of the KEGG metabolic pathway, and the horizontal coordinate is the proportion of differential metabolites to the total metabolites in the pathway; the larger the enrichment ratio, the greater the enrichment.

Table 1. Number of metabolites significantly different between monoculture and co-culture of the two strains

Ionic Category	<i>B. xylanisolvans</i> Y-11			<i>B. longum</i> y37		
	Differential metabolite	Up	Down	Differential metabolite	Up	Down
Negative ions	132	62	70	43	10	32
Positive ions	135	35	100	21	8	13

y37 in monoculture and co-culture were different, in which the pathways enriched in monoculture of both strains were similar to those enriched in *B. xylanisolvans* Y-11 before and after co-culture, and *B. longum* y37 may have a greater influence on the changes of metabolites in co-culture.

Table 2. Categories of metabolites that significantly different between monoculture and co-culture of the two strains

Metabolite Category	<i>B. xylanisolvans</i> Y-11	<i>B. longum</i> y37
Nucleotides and derivatives	18	8
Nitrile	1	0
Quinoline	1	0
Zine	1	0
Acid radical	2	0
Sugar	3	0
Hydrocarbons and derivatives	35	15
Salts	5	1
Oxides	1	0
Organic acids and esters	112	20
Heterocyclic compounds	6	1
Organic alkali	19	0
Amino acids and derivatives	0	11

Validation experiments of growth inhibition of co-culture metabolites

Effect of different additives on the growth of bacterial strains

The results showed (Table 3, Table 4) that succinic acid exhibited significant inhibition of bacterial growth at 0.01 g/L and 0.05 g/L at 0 h, and 0.03 g/L and 0.05 g/L at 12 h and 24 h. Cycloserine showed significant inhibition of bacterial growth at 0.05 g/L at 0 h, two concentrations of 0.01 g/L and 0.05 g/L at 12 h, and all three concentrations (0.01 g/L, 0.03 g/L and 0.05 g/L) at 24 h. Creatinine at a concentration of 0.05 g/L at 0 h and 0.01 g/L at 12 h showed significant inhibition of bacterial growth. Serine at 0.01 g/L at 12 h and proline at 0.05 g/L at 0 h showed significant inhibition of bacterial growth. As shown above, the growth of both strains was significantly slower after the addition of these compounds, with a delay in the onset of the logarithmic phase and a decrease in the overall OD_{600 nm} value compared to the non-additive. Among them, cycloserine and succinic acid showed significant differences from the other additives, inhibiting the growth of both strains of bacteria significantly, with succinic acid showing an increasing concentration effect, the higher the concentration the stronger the inhibitory effect on the bacteria.

before and gradually declined with the dose of addition.

Discussion

The results showed that the types and contents of the metabolites in co-cultured strains changed significantly, among which hydrocarbons and their derivatives, organic acids and esters were the main differential metabolites, while the metabolic amounts of the two strains were significantly affected by co-culture as well, and the effect on the metabolism of *B. xylanisolvans* Y11 was larger than that of *B. longum* y37. *B. xylanisolvans* Y-11 metabolized substances with a predominantly positive charge after ionization, opposite to those metabolized by *B. longum* y37.

Both strains may be affected by cycloserine and succinic acid causing their growth to be inhibited at a slower rate, and it was hypothesized that the decrease in pH may be one of the reasons for the inhibition by succinic acid. And most of the compounds tested were present only in *B. longum* y37 metabolites, which consisted with previous metabolomic assays that the metabolite content of the strains was closer to that of *B. longum* y37 after co-culture, a strain that played a major role and whose metabolites greatly influenced the growth of *B.*

Table 3. Variation of OD_{600 nm} of *B. longum* y37 at different time with different additive concentrations

Additives	0 h			12 h			24 h		
	0.01 g/L	0.03 g/L	0.05 g/L	0.01 g/L	0.03 g/L	0.05 g/L	0.01 g/L	0.03 g/L	0.05 g/L
Threonine	0.09 ± 0	0.09167 ± 0	0.09 ± 0	0.26 ± 0.01	0.25 ± 0.02	0.24 ± 0.04	0.68 ± 0.01	0.68 ± 0.01	0.69 ± 0.02
Valine	0.09 ± 0	0.09 ± 0.01	0.1 ± 0	0.27 ± 0.03	0.27 ± 0.01	0.28 ± 0.04	0.68 ± 0.03	0.71 ± 0.05	0.73 ± 0.05
Succinic acid	0.09 ± 0*	0.09 ± 0	0.08 ± 0*	0.16 ± 0.01	0.09 ± 0*	0.08 ± 0*	0.68 ± 0.02	0.72 ± 0*	0.74 ± 0*
Serine	0.09 ± 0	0.09 ± 0.01	0.08 ± 0	0.15 ± 0*	0.25 ± 0.01	0.2 ± 0.02	0.45 ± 0.03	0.66 ± 0.1	0.68 ± 0.06
Cycloserine	0.11 ± 0.01	0.12 ± 0.02	0.14 ± 0*	0.13 ± 0*	0.13 ± 0.01	0.12 ± 0.02*	0.29 ± 0.01*	0.28 ± 0.05*	0.18 ± 0*
Creatinine	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0*	0.16 ± 0.02*	0.23 ± 0.02	0.19 ± 0.02	0.64 ± 0.04	0.71 ± 0.01	0.61 ± 0.05
Proline	0.09 ± 0	0.09 ± 0.01*	0.09 ± 0.01	0.21 ± 0.01	0.24 ± 0.02	0.24 ± 0.04	0.64 ± 0.01	0.66 ± 0.04	0.64 ± 0.03
Betaine	0.09 ± 0	0.09 ± 0	0.1 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.66 ± 0.05	0.65 ± 0.1	0.64 ± 0.05
CK	0.1 ± 0			0.24 ± 0.01			0.64 ± 0.05		

* indicates a significant difference between this treatment and the CK group ($P < 0.05$)

Effect of different additives on pH of fermentation broth of bacterial strains

The pH of the medium changed to varying degrees with the addition of different compounds range from 4.55 to 7.27 (table 4 and table 5). The medium with the addition of all substances except succinic acid was alkaline, while the pH of the medium with succinic acid decreased significantly compared with that

xylanisolvans Y- 11.

In this study, co-culture, as opposed to monoculture, changed the original microbial growth trends as well as metabolite species and content, which provided a reference for studying the drive of simple changes in the environment on complex physiological behaviors in bacterial communities. Of course, the culture media still had a certain difference from the intesti-

Table 4. variation of OD_{600 nm} of *B. xylanisolvens* Y-11 at different time with different additive concentrations

Additives	0 h			12 h			24 h		
	0.01 g/L	0.03 g/L	0.05 g/L	0.01 g/L	0.03 g/L	0.05 g/L	0.01 g/L	0.03 g/L	0.05 g/L
Threonine	0.09 ± 0	0.09167 ± 0	0.09 ± 0	0.26 ± 0.01	0.25 ± 0.02	0.24 ± 0.04	0.68 ± 0.01	0.68 ± 0.01	0.69 ± 0.02
Valine	0.09 ± 0	0.09 ± 0.01	0.1 ± 0	0.27 ± 0.03	0.27 ± 0.01	0.28 ± 0.04	0.68 ± 0.03	0.71 ± 0.05	0.73 ± 0.05
Succinic acid	0.09 ± 0	0.09 ± 0	0.08 ± 0	0.16 ± 0.01	0.09 ± 0	0.08 ± 0	0.68 ± 0.02*	0.72 ± 0*	0.74 ± 0*
Serine	0.09 ± 0	0.09 ± 0.01	0.08 ± 0	0.15 ± 0	0.25 ± 0.01*	0.2 ± 0.02	0.45 ± 0.03	0.66 ± 0.1	0.68 ± 0.06
Cyclose-rine	0.11 ± 0.01	0.12 ± 0.02	0.14 ± 0	0.13 ± 0	0.13 ± 0.01	0.12 ± 0.02	0.29 ± 0.01*	0.28 ± 0.05*	0.18 ± 0*
Creatinine	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0	0.16 ± 0.02	0.23 ± 0.02	0.19 ± 0.02	0.64 ± 0.04	0.71 ± 0.01	0.61 ± 0.05
Proline	0.09 ± 0	0.09 ± 0.01	0.09 ± 0.01	0.21 ± 0.01	0.24 ± 0.02	0.24 ± 0.04	0.64 ± 0.01	0.66 ± 0.04	0.64 ± 0.03
Betaine	0.09 ± 0	0.09 ± 0	0.1 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.66 ± 0.05	0.65 ± 0.1	0.64 ± 0.05
CK	0.1 ± 0			0.24 ± 0.01			0.64 ± 0.05		

* indicates a significant difference between this treatment and the CK group ($P < 0.05$)

nal environment and could not fully reflect the changes *in vivo*. The actual metabolism of the strain *in vivo* may be influenced

one of the potential causes of influence. And the conditions of the co-culture system can be optimized in the future.

Table 5. pH changes of *B. longum* y37 at different time with different additive concentrations

Additives	0 h			12 h			24 h		
	0.01 g/L	0.03 g/L	0.05 g/L	0.01 g/L	0.03 g/L	0.05 g/L	0.01 g/L	0.03 g/L	0.05 g/L
Threonine	6.94 ± 0	6.94 ± 0	6.92 ± 0.01*	6.37 ± 0**	6.38 ± 0**	6.41 ± 0*	5.26 ± 0**	5.24 ± 0**	5.22 ± 0**
Valine	6.9 ± 0	6.9 ± 0	6.9 ± 0	6.27 ± 0*	6.23 ± 0**	6.25 ± 0*	5.26 ± 0**	5.29 ± 0**	5.22 ± 0**
Succinic acid	6.19 ± 0**	5.03 ± 0**	4.55 ± 0**	5.92 ± 0**	5.03 ± 0**	4.55 ± 0*	5.33 ± 0**	5.13 ± 0**	4.68 ± 0**
Serine	6.78 ± 0**	6.81 ± 0**	6.86 ± 0*	6.5 ± 0**	6.29 ± 0	6.55 ± 0*	5.48 ± 0	5.18 ± 0	5.2 ± 0
Cyclose-rine	7 ± 0**	7.19 ± 0.01**	7.39 ± 0.01**	6.79 ± 0**	7 ± 0**	7.27 ± 0*	6.06 ± 0**	6.44 ± 0**	7.1 ± 0**
Creatinine	6.92 ± 0	6.96 ± 0	6.95 ± 0	6.43 ± 0.01**	6.56 ± 0.01**	6.64 ± 0*	5.35 ± 0**	5.34 ± 0.03*	5.42 ± 0**
Proline	6.92 ± 0	6.89 ± 0	6.91 ± 0	6.33 ± 0	6.19 ± 0.01**	6.26 ± 0*	5.23 ± 0**	5.24 ± 0**	5.24 ± 0**
Betaine	6.92 ± 0	6.91 ± 0	6.92 ± 0.01**	6.3 ± 0	6.34 ± 0*	6.2 ± 0*	5.33 ± 0**	5.27 ± 0**	5.29 ± 0**
CK	6.91 ± 0.01			6.31 ± 0			5.18 ± 0		

* indicates a significant difference between this treatment and the CK group ($P < 0.05$), ** indicates a highly significant difference between this treatment and the CK group ($P < 0.01$)

by other flora as well as the regulation of the whole complex network, so the situation will be more complex and there may be multiple mechanisms of influence, and the present study is

The present study strengthened our earlier macro-transcriptome prediction that there existed an interlocking effect between various genera of microorganisms, especially the dom-

Table 6. pH changes of *B. xylanisolvans* Y-11 at different time with different additive concentrations

Additives	0 h			12 h			24 h		
	0.01 g/L	0.03 g/L	0.05 g/L	0.01 g/L	0.03 g/L	0.05 g/L	0.01 g/L	0.03 g/L	0.05 g/L
Threonine	6.91 ± 0**	6.91 ± 0**	6.9 ± 0**	6.24 ± 0	6.31 ± 0**	6.23 ± 0*	5.26 ± 0**	5.31 ± 0**	5.34 ± 0**
Valine	6.92 ± 0**	6.91 ± 0**	6.91 ± 0**	6.48 ± 0**	6.29 ± 0**	6.37 ± 0**	5.2 ± 0**	5.3 ± 0**	5.29 ± 0**
Succinic acid	5.57 ± 0**	5.01 ± 0.01**	4.29 ± 0.01**	5.7 ± 0.01**	5.13 ± 0**	4.64 ± 0.01**	5.49 ± 0**	5.28 ± 0**	4.71 ± 0**
Serine	6.88 ± 0**	6.9 ± 0**	6.88 ± 0**	6.11 ± 0**	6.63 ± 0**	6.24 ± 0.01	5.24 ± 0**	5.46 ± 0**	5.22 ± 0.01**
Cycloserine	7.13 ± 0**	7.19 ± 0**	7.35 ± 0.01**	6.72 ± 0**	6.98 ± 0**	7.28 ± 0**	5.56 ± 0**	6.08 ± 0**	7.05 ± 0**
Creatinine	6.95 ± 0.01**	6.95 ± 0**	6.96 ± 0.01**	6.37 ± 0**	6.46 ± 0**	6.26 ± 0	5.29 ± 0**	5.34 ± 0**	5.33 ± 0**
Proline	6.83 ± 0.01	6.94 ± 0**	6.91 ± 0**	6.17 ± 0**	6.21 ± 0*	6.27 ± 0.01	5.24 ± 0**	5.27 ± 0**	5.17 ± 0**
Betaine	6.84 ± 0	6.75 ± 0**	6.91 ± 0**	6.22 ± 0	6.26 ± 0	6.26 ± 0	5.21 ± 0**	5.22 ± 0**	5.23 ± 0**
CK	6.81 ± 0			6.25 ± 0			4.99 ± 0		

* indicates a significant difference between this treatment and the CK group ($P < 0.05$), ** indicates a highly significant difference between this treatment and the CK group ($P < 0.01$)

inant genus in the intestine, which more easily affected the structure of the intestinal flora. We have found in our previous study that the structure of intestinal flora in patients with calcium oxalate kidney stones is significantly different from that of healthy ones (Liu et al., 2020). Our preliminary 16s rRNA gene sequencing results showed that *Bifidobacterium* and *Bacteroides* were the predominant genera in healthy individuals as well as in patients with renal calculi, especially *Bacteroides* which was the second most predominant genus accounting for about 10%. Other studies have also shown that *Bifidobacterium longum*, a genus with high oxalate degrading activity, reduces intestinal oxalate absorption (Mogna et al., 2014) and attenuates inflammation associated with oxalate accumulation by stimulating the production of cytokines such as IL-4, IL-10, IFN- γ and IL-12p70 (Giardina et al., 2014). The results of this study suggest that the dominant intestinal microorganisms, represented by *Bifidobacterium longum* and *Bacteroides* Xylanolytic, may influence the formation of kidney stones by mutually inhibiting the structure of the intestinal flora and the formation of their own metabolites, which will be an essential direction for our future attention.

Conclusion

In this study, we explored the interaction mechanism between *Bifidobacterium longum* and *Bacteroides*. *xylanisolvans* through co-culture technique and concluded that both strains may be affected by cycloserine and succinic acid, leading to their growth inhibition and slowdown, where pH reduction may be the main reason for succinic acid to inhibit the growth of both strains. This study will contribute to a better understanding of the impact of intestinal microorganisms and their metabolites on the structure of the flora and the onset and pro-

gression of the disease. This work not only serves as a guide for the prevention and treatment of gut microbial-associated disease, but also provides novel perspectives for exploring the mechanisms of disease formation in the future.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Qun Sun and Lei Tian designed the research work. Lei Tian, Dongmei Luo, Rui Li, and Pengrui Jiao conducted the experiments, analyzed the data and prepared the manuscript. Dongmei Luo, Zhiwei Zhou, Lei Tian and Qun Sun critically reviewed and edited the manuscript. All authors had full access to the final version of the report and agreed to the submission.

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