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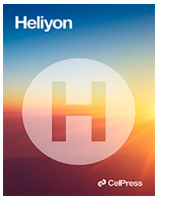

Research article

Characterization and comparison of gut microbiota in patients with acute pancreatitis by metagenomics and culturomics

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ABSTRACT

Acute pancreatitis (AP) is a common gastrointestinal disorder with a high mortality rate. This study sought to identify the microbial community structure in patients with AP using metagenomics and culturomics. Compared to healthy controls, patients with AP exhibited a significant decrease in alpha diversity; a higher abundance of *unclassified Enterococcus* species (sp), *Enterococcus faecium*, and *Enterococcus faecalis*; and a lower abundance of *Eubacterium rectale*. A total of 336 isolates from 25 genera and 44 species were obtained by sample cultivation. The dominant species identified in patients with AP were *Enterococcus faecium* and *Klebsiella grimontii*, whereas those in the healthy controls were *Enterococcus faecium*, *Escherichia coli*, and *Bacteroides faecis*. Our research has contributed to the expanded understanding of the genome, diversity, and function of the intestinal microbiota in patients with AP and provided some reference for selecting culture medium and sample processing methods.

1. Introduction

Acute pancreatitis (AP) is among the most common digestive diseases requiring acute hospitalization, with a global incidence rate of 34 per 100,000 and a mortality rate ranging from 1 % to 5 % [1]. However, as living standards improve and dietary habits change, the incidence of AP continues to rise each year. According to the revision of the Atlanta classification in 2012 [2], AP can be classified into mild acute pancreatitis (MAP), moderately severe acute pancreatitis (MSAP), and severe acute pancreatitis (SAP). Most patients with AP have a mild disease course, but approximately 20 % progress to SAP, which is associated with severe complications and high mortality rates [3]. Septic shock and multiple organ dysfunction syndrome due to infection are important causes of death in patients

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with AP [4]. The pathogenesis and pathophysiological processes of AP are complex and not fully understood. They are closely linked to trypsinogen activation, endoplasmic reticulum stress, unfolded protein response, impaired autophagy, ductal cell dysfunction, inflammatory cascades, gene mutations, triglyceride hydrolysis, intestinal mucosal barrier damage, and bacterial translocation [5].

The intestinal microbiota, a vital element of the intestinal ecosystem, can safeguard the intestinal barrier and regulate the host's immune system and metabolism [6]. Recent studies have suggested that patients with AP are often accompanied by dysbiosis of intestinal microflora and disruptions in intestinal microecology, leading to a decrease in the diversity of intestinal microflora and an imbalance between commensal and pathogenic flora [7–9]. Dysbiosis can worsen the severity of AP, creating a vicious circle [10]. Given the close relationship between gut microbiota dysbiosis and AP, interventions targeting gut microbiota and relevant metabolites may serve as useful therapeutic strategies. Prebiotics, probiotics, antibiotics, and fecal transplantation of microbiota have shown promise in modulating the gut microbiota and improving outcomes in patients with AP [11].

However, the interaction between the intestinal microbiota and AP remains unclear, and the clinical effectiveness, safety, and risks of new treatments based on the gut microbiota remain controversial. Therefore, further research is needed to elucidate the underlying mechanisms and guide the development of novel therapeutic strategies. Previous work from our team has shown that the structure and function of the gut microbiota in patients with various degrees of AP changed significantly [12], and that some intestinal flora were strongly correlated with the progression, development, and poor prognosis of AP [13,14]. Previous studies have focused on investigating the characteristics of the intestinal microbiota using 16S rRNA and metagenome sequencing technologies, but these have certain limitations. For example, some sequences cannot be classified into defined taxonomic groups via sequence similarity searches, and it is difficult to obtain functional strains for deep analysis and practical applications.

Culturomics is a culturing approach that combines a diverse repertoire of culture conditions with rapid identification via matrix-assisted laser desorption ionization time-of-flight mass spectrometry and 16S rRNA sequencing [15]. Although about 80 % of gut bacteria are unknown, culturomics expands our knowledge of the bacteria colonizing the human gut microbiome and can be used to identify and distinguish bacterial isolates, reducing the unrecognized microorganisms in metagenomics analysis [16]. This approach is crucial for understanding the differences in abundantly present bacteria, determining their pathogenic or probiotic properties, and utilizing the obtained strains for *in vitro* and *in vivo* experiments, which are essential for studying diseases and validating potential therapeutics. No studies have utilized culturomics to explore the intestinal microbiota in patients with AP.

In this study, we aimed to investigate the diversity and structural characteristics of the intestinal microbiota in AP through metagenomics and culturomics. We aimed to identify potential therapeutic targets for this disease and explore the underlying mechanisms.

2. Materials and methods

2.1. Clinical trial design and sampling

Rectal swabs were collected from 12 consecutive patients (Fig. S1) with AP and 12 healthy controls manually matched with age and gender at Peking Union Medical College Hospital, Beijing, China, between August 2021 and February 2022. The inclusion criteria were as follows: (1) a diagnosis of AP according to the 2012 revised Atlanta criteria [2], and (2) an individual must be present in the hospital within 24 h after the onset of illness. The exclusion criteria were as follows: chronic pancreatitis, immunosuppressive disease, inflammatory bowel disease, cancer, irritable bowel syndrome, gastroenteritis, narcotizing enterocolitis, and use of antibiotics, probiotics, laxatives, or Chinese herbs within two months of enrollment. A written informed consent was obtained from all subjects before any experiment was conducted, following applicable guidelines and regulations. The swab was inserted approximately 5 cm into the rectum and rotated 360° while removing it. There were few specimens available collected from patients by one rectal swab, which was difficult to meet the needs of metagenomic sequencing, while taking more rectal swabs might lead to rectal mucosal damage. Therefore, six to eight rectal swabs were collected at one time point soon after hospital admission and immediately after study consent. After collection, six swabs were frozen at −80 °C for subsequent metagenomic analysis which was performed at the same time and with the same company, and the others were kept in an anaerobic gas bag and rapidly transferred to the laboratory for cultivation. Ethical approval was obtained from the Institute of Laboratory Animal Sciences Ethics Committee, Chinese Academy of Medical Sciences & Peking Union Medical College (Identifier: SL2022001, Date of approval: April 19, 2022. Period of validity: April 2022 to June 2022).

2.2. DNA extraction and metagenomic sequencing

Microbial genomic DNA was extracted from fecal samples using the bead-beating method based on a previously described protocol [17]. Agarose gel electrophoresis was performed to determine the concentration and purity of the extracted DNA. Bacterial DNA was fragmented to an average size of about 300 bp using a Covaris M220 sonicator (Covaris). After processing, the DNA fragments were subjected to end repair, A-tail addition, sequencing junction addition, purification, polymerase chain reaction (PCR) amplification, and other steps to complete the paired-end library preparation using a TruSeq DNA sample prep kit (Illumina, United States). Inter-sizes were detected using the Agilent 2100 library, and the samples were diluted to 2 ng/μL. After the insert size was as expected, the effective concentration of the library was accurately quantified (the effective concentration of the library >2 nM) to ensure the quality of the library using the Q-PCR method. Qualified libraries were sequenced using the Illumina NovaSeq 6000 after pooling according to the effective concentration and expected data volume.

2.3. Assembly and annotation

Metagenomic data were assembled using MEGAHIT [18] (version 1.1.2) via succinct de Bruijn graphs. The final assembly consisted of contigs with 300 bp or greater lengths. Open reading frame prediction was performed with MetaGene [19]. DIAMOND [20] was used to align representative sequences of the NCBI non-redundant gene catalog to the NR database located at NCBI, and the relative abundance of species was calculated at the kingdom, phylum, and class levels. The functional annotation of unigenes was conducted by BLAST searching against public databases, including the Cluster of Orthologous Groups (COG) [21], KEGG, CAzY, ARDB, CARD, and VFDB.

2.4. Cultivation and identification of intestinal flora

The rectal swabs were immediately transferred into an anaerobic glove box (Belle Technology), which contained 90 % N_2, 5 % H_2, and 5 % CO_2, and soaked in 5 mL sterile anaerobic phosphate-buffered saline (PBS) (pH 7, containing cysteine at 1 %) to prepare the bacterial suspension. From the prepared stock solution, 1 mL was separately withdrawn and further processed using three different methods: treatment with 70 % alcohol, incubation at 65 °C for 30 min, and incubation at 80 °C for 10 min. The four different solutions were tenfold diluted with sterile anaerobic PBS to obtain 10−1 to 10−7 serial dilutions and spread on yeast extract, casein hydrolysate, and fatty acid (YCFA) medium [21] and Modified Gifu Anaerobic Medium (MGAM) [22] agar plates supplemented with sterile sheep blood (5 %) and rumen fluid (10 %), for either aerobic or anaerobic incubations at 37 °C [23,24]. Single bacterial colonies were isolated and cultivated in a liquid medium. After incubation, bacterial cells were harvested by centrifugation, washed once with PBS, and lysed with lysis buffer. Amplification of the 16S rRNA gene was performed using universal primers, 27F as the forward primer and 1492 as the reverse primer [25]. The isolated bacterial strains were identified by 16S rRNA sequencing and stored at −80 °C.

2.5. Bioinformatic and statistical analysis

R software (version 4.0.3) was used to analyze the diversity of microbial communities. The Shannon index for alpha diversity analyses was calculated using the vegan package (version 2.5-7) and the reshape2 package (version 1.4.4) of R software. The vegan package was utilized for PCoA analysis and visualization, and QIIME software was used to calculate the beta diversity distance matrix. The Kruskal-Wallis rank-sum test was used to analyze significant differences between groups for alpha and beta diversity. Based on the relative abundance, stacked bar plots and heatmaps were used to visualize the microbial community structure at the phylum, family, and genus levels. Differential abundance testing was conducted using Multivariable Association with Linear Models (MaAsLin2). Statistical analyses of the clinical characteristics were conducted using Statistical Package for Social Sciences (SPSS) software (version 25.0). The data are expressed as mean ± standard deviation (SD) for continuous variables with normal distribution, as median (Interquartile Range [IQR]) for continuous variables with abnormal distribution, and as number (percentages) for categorical variables. The statistical significance of categorical variables was assessed using the Chi-square or Fisher test, while the *t*-test or non-parametric Kruskal-Wallis test was employed for continuous variables. A two-sided *p*-value less than 0.05 was deemed statistically significant.

3. Results

3.1. Clinical characteristics

A total of 12 patients with AP were enrolled in this study, and 12 healthy individuals matched for sex, age, and body mass index (BMI) served as the control group (CON), as indicated in Table 1.

According to clinical severity, these patients were separated into three groups: MAP, MSAP, and SAP. The characteristics of the three groups in this study are shown in Table 2.

3.2. Microbiota analysis by metagenomics

3.2.1. Overall differences

A total of 144 rectal swabs from 24 participants (a mean of six swabs per sample) were collected and sent for sequencing. No sample was discarded due to poor sequencing quality. The final non-redundant dataset contained 2623189 genes with an average length of 695.84 bp. Based on the results of species annotation, Venn diagram analysis revealed that 9590 and 11761 species were observed in

Table 1
The basic information of cases and control groups.

Variables	AP group (n = 12)	CON group (n = 12)	*p*
Age (y), mean (SD)	34.92 ± 9.31	29.50 ± 9.74	0.178
Male, n (%)	4 (33.33)	4 (33.33)	1.000
BMI (kg/m^2), mean (SD)	25.73 ± 5.70	22.29 ± 2.76	0.780

SD, standard deviation; BMI, body mass index.

Table 2
The demographic and clinical characteristics of subgroups.

Variables	MAP (n = 4)	MSAP (n = 4)	SAP (n = 4)	*p*
Age (y), mean (SD)	29.75 ± 11.70	34.25 ± 6.65	40.75 ± 7.41	0.263
Male, n (%)	1 (25.00)	2 (50.00)	1 (25.00)	1.000
BMI (kg/m^2)	25.98 ± 7.71	26.20 ± 6.41	25.02 ± 4.17	0.961
Smoking, n (%)	1 (25.00)	1 (25.00)	0 (0.00)	1.000
Drinking, n (%)	0 (0.00)	3 (75.00)	1 (25.00)	0.200
Comorbid abnormalities, n (%)				
Hypertension	2 (50.00)	1 (25.00)	1 (25.00)	1.000
Diabetes	3 (75.00)	2 (50.00)	1 (25.00)	0.766
Fatty liver	3 (75.00)	3 (75.00)	3 (75.00)	1.000
Laboratory examinations				
Amylase (U/L)	438.0 (232.8–920.0)	431.0 (244.8–540.0)	817.0 (441.0–1165.3)	0.298
Lipase (U/L)	872.5 (505.8–6797.5)	3877.0 (1150.0–6942.3)	3827.5 (2396.3–6181.3)	0.472
Triglyceride (mmol/L)	24.64 (11.42–42.19)	8.70 (2.22–32.37)	13.28 (8.91–17.43)	0.368
CRP (mg/L)	95.0 (65.3–169.0)	150.0 (124.0–228.5)	152.5 (140.5–217.8)	0.263
Etiology, n (%)				
Biliary	1 (25.00)	1 (25.00)	0 (0.00)	0.709
Hypertriglyceridemia	3 (75.00)	2 (50.00)	3 (75.00)	
Alcohol consumption	0 (0.00)	1 (25.00)	1 (25.00)	
APACHE II, median (IQR)	4.0 (3.0–5.8)	4.5 (2.3–6.0)	8.5 (6.0–11.0)	0.069
SOFA score, median (IQR)	1.0 (0.3–2.5)	2.0 (1.3–2.0)	6.5 (3.0–7.8)	0.051
Balthazar score E, n (%)	0 (0.00)	0 (0.00)	2 (50.00)	0.273
Local complications, n (%)				
APFC	0 (0.00)	4 (100.00)	2 (50.00)	0.006
Pancreatic pseudocyst	0 (0.00)	0 (0.00)	1 (25.00)	1.000
ANC	0 (0.00)	0 (0.00)	1 (25.00)	1.000
WON	0 (0.00)	0 (0.00)	0 (0.00)	1.000
Infected necrosis	0 (0.00)	0 (0.00)	0 (0.00)	1.000
Systematic complication, n (%)				
SIRS	0 (0.00)	3 (75.00)	4 (100.00)	1.000
ARDS	0 (0.00)	0 (0.00)	3 (75.00)	0.055
AKI	0 (0.00)	1 (25.00)	2 (50.00)	0.709
Shock	0 (0.00)	0 (0.00)	2 (50.00)	0.273
ACS	0 (0.00)	0 (0.00)	1 (25.00)	1.000
Liver damage	0 (0.00)	1 (25.00)	1 (25.00)	1.000
Myocardial injury	0 (0.00)	0 (0.00)	1 (25.00)	1.000
Disorder of consciousness	0 (0.00)	0 (0.00)	1 (25.00)	1.000
Sepsis	0 (0.00)	0 (0.00)	1 (25.00)	1.000
Bowel obstruction	0 (0.00)	0 (0.00)	1 (25.00)	1.000
Outcome				
Organ failure, n (%)	0 (0.00)	1 (25.00)	4 (100.00)	0.030
Organ failure duration (h), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–12.0)	144.0 (48.0–402.0)	0.009
ICU, n (%)	0 (0.00)	1 (25.00)	3 (75.00)	0.200
ICU stay (d), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–7.5)	14.5 (2.3–23.8)	0.086
Hospital stays (d), median (IQR)	14.5 (11.3–20.0)	9.5 (7.5–13.0)	26.0 (21.3–29.3)	0.017
Death, n (%)	0 (0.00)	0 (0.00)	0 (0.00)	1.000

SD, standard deviation; BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; APACHE II, Acute Physiology and Chronic Health Evaluation II score; SOFA, Sequential Organ Failure Assessment score; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; APFC, acute peripancreatic fluid collection; ANC, acute necrotic accumulation; WON, walled-off necrosis; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; ACS, abdominal compartment syndrome; SIRS, systemic inflammatory response syndrome; ICU, intensive care unit.

the AP and CON groups, respectively, and these two groups shared 7486 species in common (Fig. S2A). In the subgroup analysis, there were 4052 common species in the three groups (Fig. S2B).

3.2.2. Gut bacterial diversity and composition

With the applied sequencing depth, the species accumulation curves of each group tended to plateau (Fig. 1A). As illustrated in Fig. 1B, the Shannon index of intestinal microflora in the AP group was significantly lower than that in the CON group ($p < 0.001$), while no significant difference was observed in the subgroup analysis of patients with AP of different severities (Fig. 1C).

The samples were analyzed based on beta diversity between groups to reflect the diversity differences among the samples. In the principal coordinate analysis (PCoA), we identified the microbiota distances based on the Bray-Curtis dissimilarity matrix. The distance between each dot represents the difference in community composition between samples. As revealed in the results, samples in the CON group clustered intensely and shifted away from the AP group ($p = 0.014$, R2 = 1.927, Fig. 1D). In contrast, there were no significant differences in the gut microbiota composition among the subgroups ($p = 0.827$, R2 = 0.774, Fig. 1E).

As illustrated in Fig. 3, we observed the proportions of different species in each group using a relative abundance histogram. At the species level, the unclassified *Enterococcus*, *Escherichia coli*, and *Enterococcus faecium* were the dominant taxa in the gut microbiota of

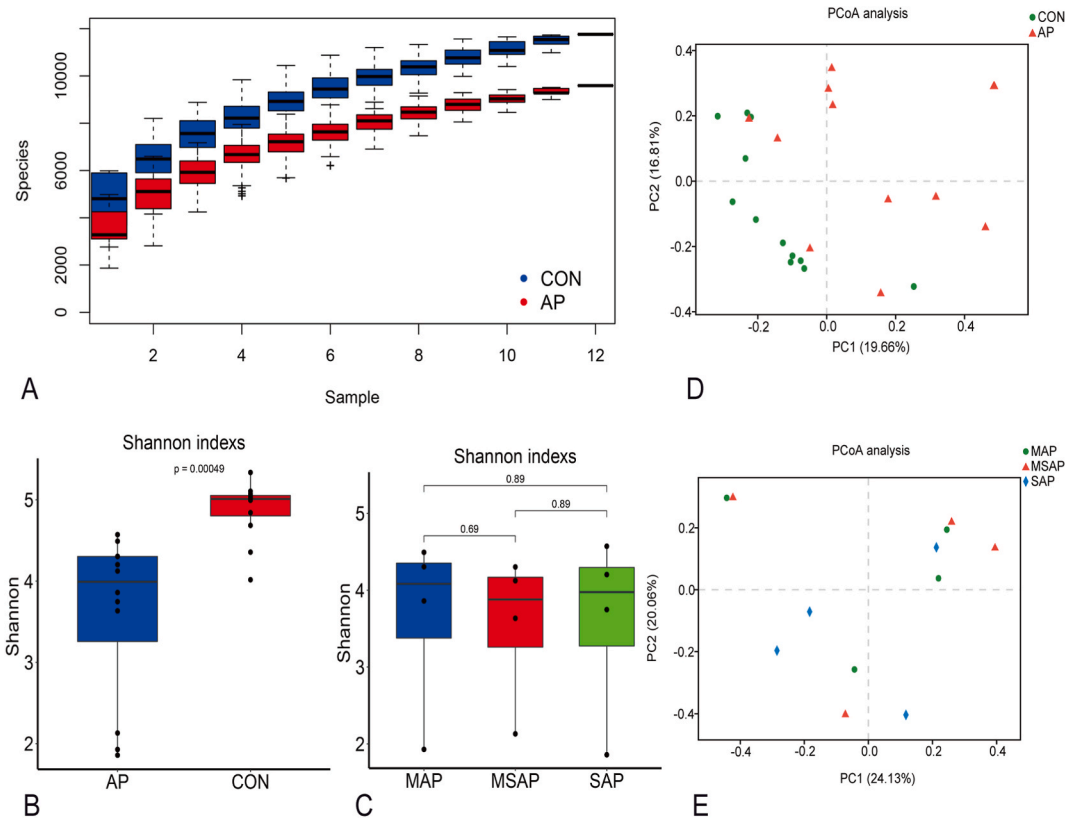

Fig. 1. Diversity analysis of the intestinal microbiota. (A) Species accumulation curve. (B) Shannon index for case and control groups. (C) Shannon index in AP groups with different severities. (D) Beta diversity in the case and control groups. (E) Beta diversity of AP groups with different severities. The PC1 and PC2 axes represent the first two principal coordinates, which explain most of the variation observed between the samples.

the AP group. In contrast, *Escherichia coli*, unclassified *Bacteroides*, and *Prevotella copri* were the specific dominant species in the control group (Fig. 2A).

In the subgroup analysis (Fig. 2B), the predominant species in the MAP group were unclassified *Enterococcus* and *Enterococcus faecium*. The dominant species for the MSAP group were the unclassified *Enterococcus*, *Enterococcus faecium*, and *Ruminococcaceae bacterium*. The dominant species in the SAP group included *Escherichia coli,* unclassified *Enterobacteriaceae*, and unclassified

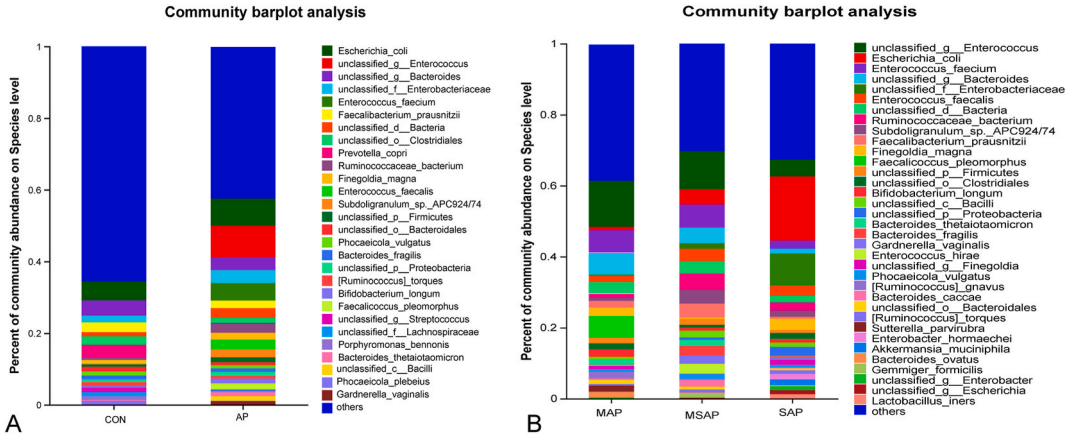

Fig. 2. Microbial community at the species level. (A) Bar plot showing the species composition in the case and control groups. (B) Bar plot showing the species composition in the AP groups with different severities. The abscissa represents the sample species proportion, and the ordinate represents the sample name. Different colors represent different species, and the length of each segment in each vertical bar represents the proportion of species.

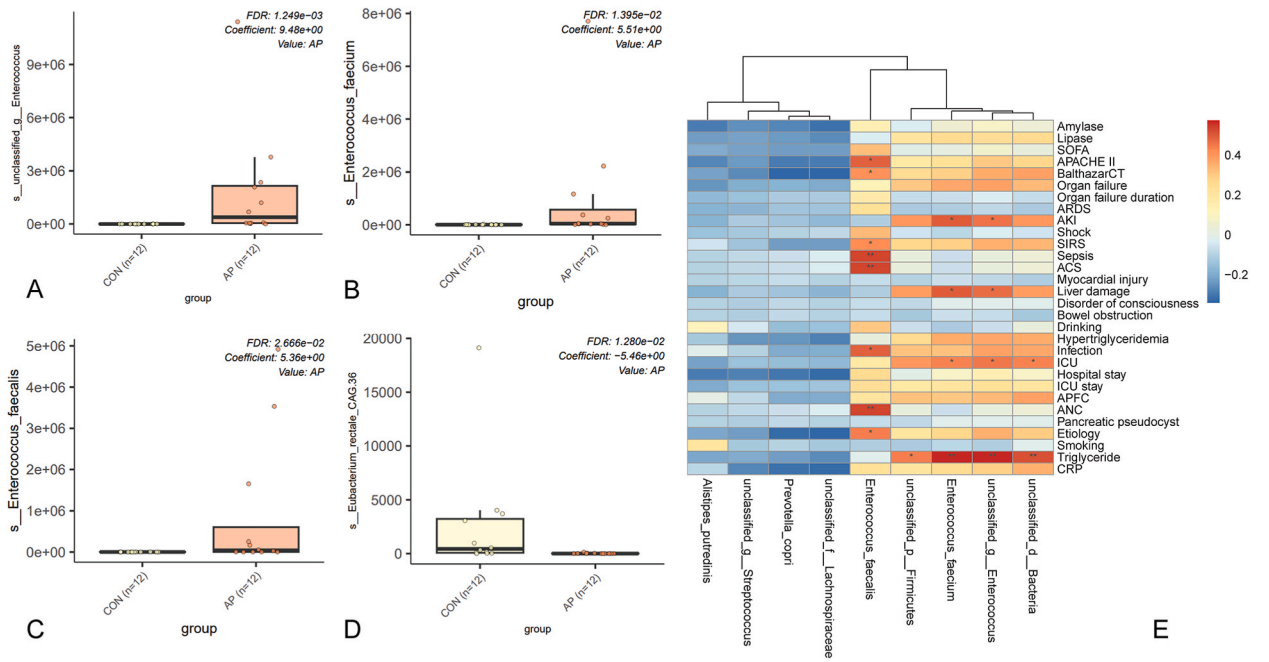

Fig. 3. Species differences in the intestinal microbiota. (A–D) Species differences between the AP and control groups by MaAsLin2 analysis. (E) Spearman correlations between differential bacterial species, clinical outcomes, and indicators of disease severity. SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation II score; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; SIRS, systemic inflammatory response syndrome; ACS, abdominal compartment syndrome; ICU, intensive care unit; APFC, acute peripancreatic fluid collection; ANC, acute necrotic accumulation; CRP, C-reactive protein. The strength of the positive (red) or negative (blue) correlation is shown by a two-color heatmap, with asterisks denoting statistical significance ($*p < 0.05$, $**p < 0.01$).

Enterococcus.

3.2.3. *Species differences reflecting the severity of AP*

A significant difference between the AP and control groups was determined using the MaAsLin2 ($FDR < 0.05$). Compared to the control group, the relative abundances of unclassified *Enterococcus* species (sp), *Enterococcus faecium*, and *Enterococcus faecalis* were significantly increased (Fig. 3A–C). In contrast, the relative abundance of *Eubacterium rectale* was significantly decreased (Fig. 3D). Pearson's correlation coefficients were used to calculate the correlation between species differences and clinical indicators, like disease severity and clinical outcomes. As illustrated in Fig. 3E, *Enterococcus faecium* and unclassified *Enterococcus* sp. had moderate to strong positive correlations with acute kidney injury (AKI), liver damage, intensive care unit (ICU) admission, and triglyceride levels.

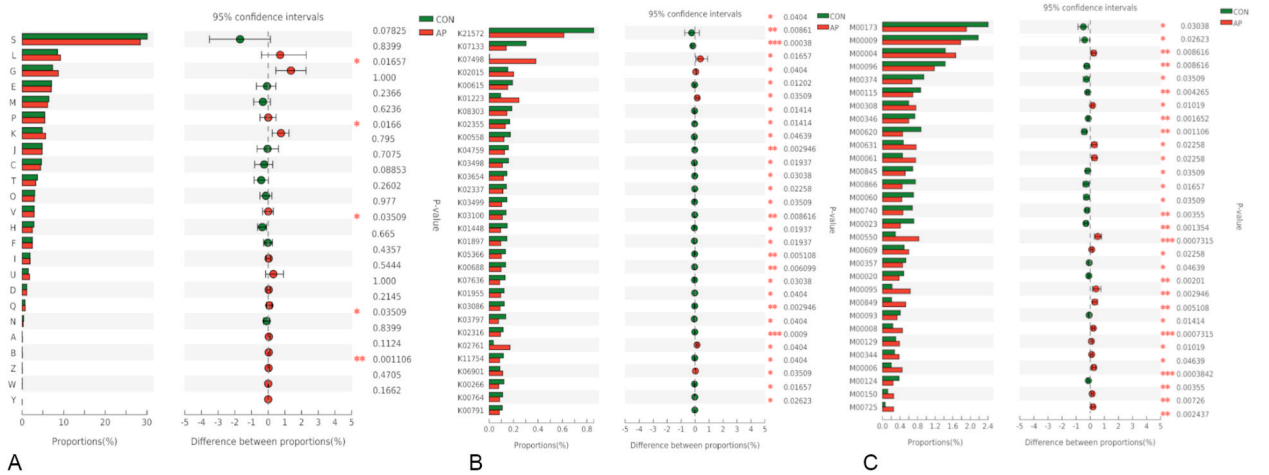

Fig. 4. Functional differences in the intestinal microbiota between the AP and control groups. (A) COG functional pathway. (B) KEGG orthologs. (C) KEGG modules. COG, Cluster of orthologous genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Enterococcus faecalis was positively correlated with sepsis, ACS (abdominal compartment syndrome), ANC (acute necrotic accumulation), SIRS (systemic inflammatory response syndrome), and infection.

3.2.4. Association of predicted microbiome functions with AP

According to the COG (Cluster of orthologous genes) and KEGG (Kyoto Encyclopedia of Genes and Genomes) databases, we categorized the differentially expressed genes based on their functional classifications. In contrast to the control group, the upregulated COG functions in the AP group included carbohydrate transport and metabolism, transcription, and cytoskeleton, while downregulated functions included coenzyme transport, metabolism, and cell motility (Fig. 4A). The upregulated genes included putative transposase, iron complex transport system permease protein, and cellobiose phosphotransferase system (PTS) EIIC (enzyme IIC component) component. In contrast, downregulated genes contained starch-binding outer membrane protein, transketolase, and U32 family peptidase (Fig. 4B). The upregulated functional modules included the pentose phosphate pathway, semi-phosphorylative Entner-Doudoroff pathway, ascorbate degradation, and cationic antimicrobial peptide resistance. In contrast, downregulated functional modules included the citrate cycle, C5 isoprenoid biosynthesis, and dicarboxylate-hydroxybutyrate cycle (Fig. 4C).

3.2.5. Differentially abundant microbial genera and species in AP and healthy individuals by culturomics

As shown in Table S1, 336 bacterial colonies were isolated from rectal swab samples collected from two patients with AP and two healthy controls. These isolates were affiliated with 4 phyla, 7 classes, 9 orders, 18 families, 25 genera, and 44 species.

The results of the isolates from patients with AP and healthy humans were merged (Fig. 5). At the genus level, the dominant genera in the case group included *Enterococcus*, *Klebsiella*, and *Paenibacillus*. In contrast, the dominant genera in the control group contained *Enterococcus*, *Bacteroides*, *Paenibacillus*, *Clostridium*, and *Escherichia*. At the species level, the dominant species in the case group included *Enterococcus faecium* and *Klebsiella grimontii*. In contrast, the dominant species in the control group contained *Enterococcus faecium*, *Escherichia coli*, and *Bacteroides faecis*.

4. Discussion

With increasing research on the intestinal microbiota in AP, increasing evidence has shown that the intestinal microbiota is vital for the development and progression of AP [11]. Gut microbiota research primarily involves multi-omics data integration based on metagenomics, transcriptomics, proteomics, metabolomics, and culturomics. Culturomics is an important complement to metagenomics in gaining a thorough insight into the gut microbiota. To the best of our knowledge, this is the first study to evaluate

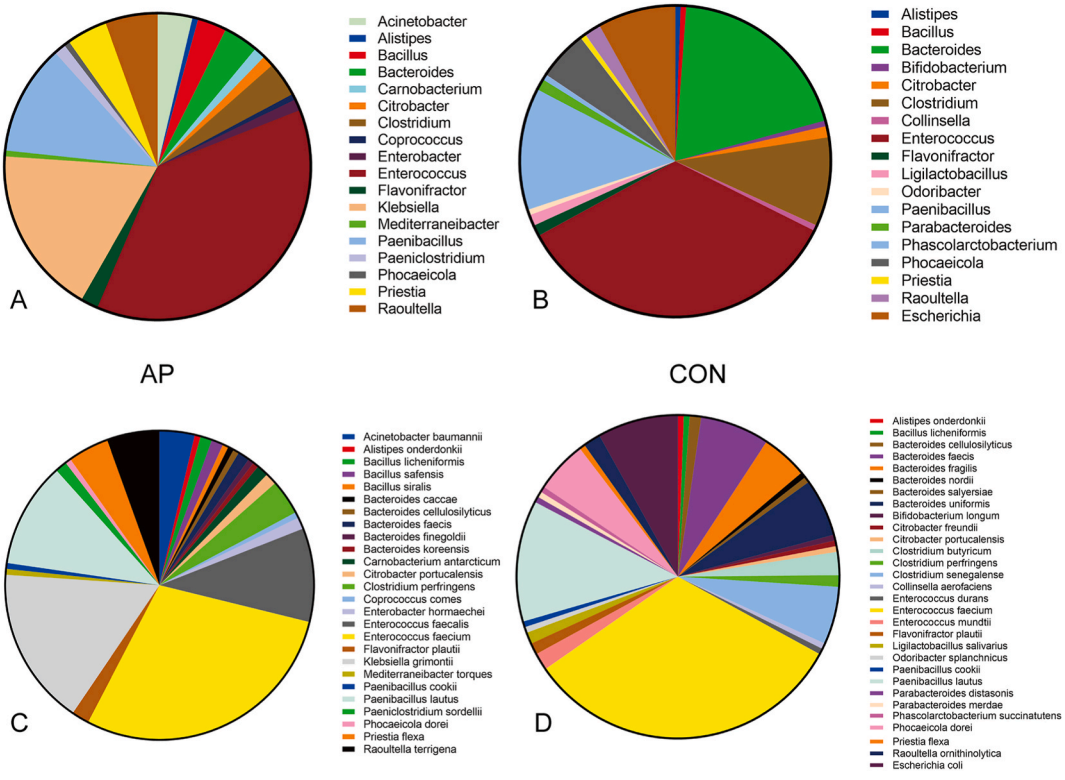

Fig. 5. Overview of cultivated bacteria from AP and control groups. (A) Genus levels in the AP group. (B) Genus levels in the control group. (C) Species level in the AP group. (D) Species levels in the control group.

intestinal microbiota diversity in patients with AP, combining metagenomics and culturomics. In our study, we analyzed the alpha and beta diversity indices to investigate the differences in intestinal microbiota between AP and CON groups. The results indicated that the alpha diversity was significantly lower in the AP group than in controls ($p < 0.001$), leading to an imbalance in the intestinal microbiota, intestinal dysfunction, and metabolic disorder, and consistent with previous studies [26]. Moreover, beta diversity analysis showed a significant difference in the microbial composition between the AP and CON groups, suggesting that AP is closely related to intestinal flora alteration.

In species composition analysis, the unclassified *Enterococcus*, *Escherichia coli*, and *Enterococcus faecium* were the dominant taxa in the gut microbiota of the AP group at the species level. In contrast, *Escherichia coli*, unclassified *Bacteroides*, and *Prevotella copri* were the specific dominant species in the control group. *Bacteroides*, *Enterococcus*, *Escherichia coli*, and *Prevotella copri* are the resident gut microflora in the human body, among which *Enterococcus* and *Escherichia coli* are opportunistic pathogens. Recent studies showed that *Enterococcus* and *Escherichia coli* are the main pathogenic bacteria causing infectious pancreatic necrosis [27], significantly associated with poor prognosis in patients with AP [28]. *Bacteroides* are dominant anaerobes of the human intestinal microflora, produce short-chain fatty acids (SCFAs), like butyrate, acetate, and propionate [29], and assist in decomposing polysaccharides to improve nutrient utilization [30]. Besides, recent evidence shows that Bacteroides play a positive role in accelerating blood vessel formation in the intestinal mucosa [31], promoting the development of the immune system [32], and maintaining the intestinal microecological balance [33]. However, the impact of *Prevotella* on human health remains controversial. Some authors believe that *Prevotella* could decrease cholesterol biosynthesis [34], break down dietary fiber, and improve glucose metabolism [35,36], while other studies have reported promoting inflammatory reactions related to *Prevotella* [37,38]. In addition, the relative abundance of opportunistic pathogens was significantly increased compared to the control group. In contrast, the relative abundances of *Eubacterium rectale* were significantly decreased. *Eubacterium rectale* is among the main butyrate producers in the gut and has anti-inflammatory functions [39]. The short-chain fatty acids are a fundamental energy source for intestinal epithelial cells and are vital for regulating energy metabolism, inflammatory responses, and intestinal barrier functions [40].

Functional predictions of different microbiota can provide information on their function in disease pathophysiology. The PTS was initially identified in *Escherichia coli* and is predominantly involved in the uptake and metabolism of carbohydrates in energy transduction processes. As research advances, the role of the PTS in various cellular processes, like metabolism regulation, transcriptional control, ion homeostasis maintenance, biofilm formation, and virulence gene expression, has been increasingly elucidated [41]. Le Bouguénec et al. [42] indicated that carbohydrate metabolism influences the virulence of pathogenic *Escherichia coli*, with PTS transport proteins serving as environmental sensors that modulate bacterial virulence. Additionally, starch-binding outer membrane proteins are crucial for most *Bacteroides* spp. to acquire starch for metabolic purposes [43]. Consistent with the findings of this study, the relative abundance of *Escherichia coli* was higher in the AP group than in the control group. In contrast, the abundance of *Bacteroides* was lower in the AP group. Functional analysis using the COG database indicated an increase in carbohydrate transport and metabolism functions, while analysis of KEGG genes revealed an upregulation of the enzyme IIC component of the fibrous disaccharide PTS system and a decrease in starch-binding outer membrane proteins. It is hypothesized that in AP, opportunistic pathogens like *Escherichia coli* may respond to changes in the external environment through the PTS, leading to the upregulation of genes involved in carbohydrate metabolism and virulence. This response may result in competition with probiotic microorganisms for survival, ultimately causing an imbalance and dysfunction within the intestinal microbiota, thereby exacerbating the progression of AP. Additionally, vitamin C (ascorbic acid) exhibits antioxidant properties by inhibiting oxidative stress, reducing lipid peroxidation, scavenging free radicals, promoting collagen synthesis, regulating immune responses, and improving inflammatory status [44]. Oxidative stress is a key factor in developing acute pancreatitis. Scott et al. [45] revealed a notable reduction in plasma vitamin C and ascorbic acid levels in patients with AP. High-dose vitamin C treatment showed promising results in managing AP [46,47]. Additionally, the AP group exhibited an increase in the ability of the intestinal microbiota to degrade ascorbic acid compared to the control group, potentially contributing to the observed decrease in plasma ascorbic acid levels in patients with AP. Moreover, the phosphoenolpyruvate phosphotransferase system and the Entner-Doudoroff pathway are prominent pathways in bacterial sugar metabolism [42], and their upregulation is linked to higher levels of opportunistic pathogens. Cationic antimicrobial peptides (CAMP) exhibit wide-ranging antimicrobial effects, and an increase in resistance to CAMP suggests increased invasiveness and virulence of opportunistic pathogens within the intestinal microbiota of individuals with AP. Virulence genes regulate bacterial virulence, and the degree of expression correlates with bacterial pathogenicity. Recent studies have demonstrated that catecholamines can stimulate the expression of virulence genes in *Escherichia coli*, suggesting that bacteria can detect alterations in their external surroundings and adapt accordingly. In the context of AP, intestinal dysfunction can result in fluctuations in intestinal osmotic pressure, pH, oxygen levels, ion concentrations, and nutrient availability. Opportunistic pathogens within the intestinal microbiota exhibit increased expression of virulence genes in response to specific stimuli, thereby facilitating disease progression. However, a more thorough examination of the precise mechanisms involved is required.

In culture-based microbiome profiling, the bacteria predominantly isolated and identified from rectal swab samples of patients with AP are primarily classified within *Enterococcus*, *Klebsiella*, and *Paenibacillus* genera. In contrast, those from rectal swab samples of healthy individuals are primarily classified within the genera *Enterococcus*, *Bacteroides, Paenibacillus*, *Clostridium*, and *Escherichia*. At the species level, the predominant species in the AP group included *Enterococcus faecium* and *Klebsiella grimontii*, whereas the predominant species in the control group were *Enterococcus faecium*, *Escherichia coli*, and *Bacteroides faecis*. The *Klebsiella* genus, a prominent opportunistic pathogen in secondary infections of patients with AP, was initially identified in infected soft tissues and has since been linked to various illnesses, including bacteremia, hemorrhagic colitis, and diabetic foot infections. *Enterococcus faecalis, Clostridium difficile*, and *Escherichia coli* are frequently encountered opportunistic pathogens, whereas *Bifidobacterium* is a prevalent probiotic in the gastrointestinal tract. *Bifidobacterium* promotes Immunoglobulin A production within the intestine and aids in

repairing the intestinal epithelial barrier and regulating inflammatory processes. Immunoglobulin A is highly concentrated in the intestines of healthy individuals and serves a vital role in safeguarding the intestinal mucosa against pathogens. Culture-based microbiome profiling demonstrated variations in the composition of intestinal microbiota between individuals with acute pancreatitis and those who are healthy, notably marked by diminished diversity and prevalence of probiotics, as well as an escalation in opportunistic pathogens among patients with AP. Besides, the isolation and characterization of three novel bacterial species underscores the potential of culture-based microbiome profiling in enriching the repository of human intestinal microbiota, thereby holding substantial implications for future research on the relationship between gut microbiota and human health. Finally, the method presented here offers numerous practical advantages. It facilitates the selective application of resources to specific intestinal flora, which is expected to provide a reference for selecting culture medium and sample processing methods.

This study has several limitations. A proportion of patients with mild acute pancreatitis did not require hospital admission, which had lower willingness to participate in this study. In the future study, we will scrupulously design experiments and collect more samples to verify the conclusion of this study.

Our data contributes to the comprehension of the genetic underpinnings of the physiology, biochemical pathways, and evolution of the isolates while also offering an initial examination of the composition of the intestinal microbiota through culturomics. However, the limited sample size and culture conditions warrant further investigation. Future research should focus on conducting larger-scale bacterial isolation and identification, more comprehensive screening of isolates exhibiting overgrowth and increased bacterial translocation *in vivo* through metabolite detection methods and confirming the effects of intestinal microbiota imbalance on the advancement of AP.

5. Conclusions

Metagenomic and culturomic data analyses demonstrated significant differences in the intestinal microbiota composition between individuals with AP and healthy controls. These distinctions include decreased microbial diversity, increased abundance of opportunistic pathogens, and reduced levels of probiotic organisms. Additionally, examination of gene functions and metabolic pathways revealed a notable upregulation of virulence-related genes and carbohydrate transport and metabolism pathways in patients with AP compared to healthy individuals. Our study enhanced the knowledge of the genome, diversity, and intestinal microbiota role in individuals with AP. These results indicate that heightened virulence of opportunistic pathogens may contribute to the development and advancement of AP. However, further research is required to determine the physiological and pathological implications of these bacteria.

CRediT authorship contribution statement

Liang Gong: Writing – original draft, Visualization, Software, Investigation, Formal analysis. **Xue Li:** Formal analysis. **Li Ji:** Formal analysis. **Guorong Chen:** Investigation. **Ziying Han:** Investigation. **Lei Su:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Data curation. **Dong Wu:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Laboratory Animal Sciences, CAMS & PUMC (Identifier: SL2022001).

Data availability statement

Data are available in a public, open access repository. Sequencing datasets have been deposited to the NCBI Sequence Read Archive under BioProject accession no. PRJNA1123460. Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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