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Investigation of the Pectin Degradation Ability of *Hominibacterium faecale* Strain SF3^T Isolated from Human Feces

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Pectin is one of the most important dietary fibers as a prebiotic to determine the composition of human gut microbiome. The study was designed to investigate the pectin degradation ability of *Hominibacterium faecale* strain SF3^T, its isolated from human feces and cultivated with different concentration pectin under anaerobic conditions in vitro. The growth ratio of strain SF3^T formed in batch culture was examined every 10 h during the 60-h incubation time using the spectrophotometer, high-performance liquid chromatography, and gas-chromatography. The pure culture of *Hominibacterium faecale* pectin degradation activity was present and commonly increases the growth value of strain SF3^T after pectin fermentation. Regarding volatile fatty acids, acetate, propionate and butyrate levels rapidly raised after 30 h of incubation. The results suggest that pectin fermentation displays the greatest contribution for human health and confirm that pectin degradation leads to the production of acetate, butyrate and propionate.

Keywords: Pectin; degradation; volatile fatty acids; prebiotic.

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1. INTRODUCTION

Pectin is a kind of polysaccharide in food additives that is indigestible by human enzymes [1]. But it can be easily digested by the interaction of human gut bacteria with the production of short-chain fatty acids (SCFAs) [2]. Human gastrointestinal tract is mediated by fraction of the microbial community [3], promote incomplete fermentative degradation processes. And contribute with human gut cells to producing of the galactorunic acids, and play an important role in gut inflammation, immunomodulation system, and drug/ nutrient interaction and have been elevated as a diet supplement for enhancing the cholesterol level, lowing blood pressure, and improving the overall gut health [4].

As indigestible substrates, including pectin could play an important roles to assess the human-gut microorganisms, and their contribution to produce galacturonic acids after digestion of pectin by human gut microbiome [5]. Former studies of the microbiome have assessed mechanisms of pectin degradation in the GIT and the impact of ingestion on the microbial community [6]. Similar studies about the physiological and pharmacology of humans have examined how pectin influences human health at the cellular level, although comprehensive efforts to link microbial metabolism of pectin in the human gut microflora that confer health benefits [7].

The biological function of pectin is to cross-link cellulose and hemicellulose fibers, providing resistance to the cell wall [8]. The human gut microbiome interacts with host physiology and condition for determination of pectin degradation ability of strain $SF3^T$ and carried out accordance with relevant guidelines and regulations and approved by the following methods and instruments.

1.1 Growth Media

Pectin from citrus peel was purchased from Chinese medicine and was composed >74% galacturonic acid because a basal medium is non-selective and supports the growth of several organisms, we selected for this study the basal medium used was peptone yeast extract (PY) containing 0.5g/L peptone 2g/L yeast extract 1g/L sodium bicarbonate 40ml/L salt solution see (DSMZ-medium104c), 0.5g/L L-cysteine as redox and 0.5ml/L resazurin as indicator and boiled 30 min under 99.99% purity of N_2 gas and autoclaved 121 ^oC for 30 min and distributed in 10 ml tubes and 50 ml serum bottles covered by rober stopper [14]. PY medium with health understanding of the mechanisms of pectin metabolisms acquisition by the human gut microbiome underpins the development of probiotic and prebiotic strategies that maximize human health [9]. While pectin acquisition by human gut Bacteroidetes species is well established, it should be emphasized that Firmicutes are more abundant in the human gut [10]. However, the mechanism by which they metabolize complex carbohydrates is less understood [11].

Previously isolated a novel strain from human feces deposited in our lab (=$CCAM730^T$) the GenBank accession number of 16SrRNA sequence (MZ297465) in NCBI website, (*Hominibacterium faecale*) belonging to Firmicute phylum whose selective growth on pectin was mediated through a tight cell-substrate interaction which is considered as a hallmark trait of primary degrading bacteria. This raised the possibility that within the human gut, there is an insufficiently characterized ecological niche for pectin-degrading bacterium species, which initiate the cascade of primary degradation by dissolving the obstructive pectin layers to expose attachment sites for heterogenous bacterial species and release oligosaccharides for utilization by secondary feeders of the microbial community.

2. MATERIALS AND METHODS

The strains isolated from human fecal samples have difficulties in transfer or expansion. There are many uncertain factors leading to a large number of strains that are not growing well and cannot meet the requirements for subsequent experiments [12]. In this study worked under anaerobic conditions and explore the bacterial cultivation and characterization with different anaerobic medium [13]. We selected pectin as a substrate with different concentration (0.5%, 1% and 2%) added in PY medium and every groups has one control and three replicates [15].

2.1 Determination of Growth Condition of Strain $SF3^T$ in PY Medium with Pectin

For determination of the growth value of strain $SF3^T$ and its pectin degradability, strain $SF3^T$ was inoculated with 10% of inoculum into 5ml tubes in PY medium containing 0.5%, 1% and

2% pectin as carbon sources and sodium thiosulfate as an electron acceptor. Every group had one control three replicates. Control group without adding any carbon sources. All samples were incubated at 37 ^{o}C for 60 hours, the growth ratio was monitored by OD600 (DU730 Beckman Coulter Germany). Collected 1ml of inoculum at various incubation time (0, 20, 40, and 60 h) all samples were after centrifugation supernatant was transferred to EP tubes for further analysis and stored at -80 ^{o}C.

2.2 Microscopic Observation of Strain SF3^{T} by Confocal Microscope

Strain SF3^{T} was incubated at 37 ^{o}C for 45h cells were collected by centrifugation at 13000 rpm for 10 min, washed twice with PBS buffer pH 7.2 dropped the cell suspension onto the slide and let it air dry, added 15µ DAPI solution to the slide [16]. Dye 5 min (Biyun Tian) flush with desterilized water air dry naturally. Add an appropriate amount of anti-fluorescence quenching agent (beyotime biotechnology), about 35µ and cover with a cover slide and use the confocal fluorescent microscope for microscopic examination.

2.3 Gas Chromatography Analysis

For analysis of the fermentation product of strain, SF3^{T} incubated at 37 ^{o}C with different concentrations of pectin in MB medium in 5ml tubes for production of H_2 and CO_2, we used the gas chromatography (GC 2010 Shimadzu Japan) to the analysis of the gas production activity of strain SF3^{T}.

2.4 VFA Concentration Analysis

Major products of microbial fermentation of different concentrations of pectin are incubated under the anaerobic condition to determine the value of different VFAs, with most abundant metabolites being acetate, propionate and butyrate [17]. The quantification of VFAs in one control, three replicates at each incubation time (0, 45, and 60 h) were transferred to new EP tubes, and aliquots were frozen at -20 oC for VFAs analysis. Before analysis, all samples were filtered through a membrane filter (pore size:0.22µ) and was analyzed using gas chromatography-mass spectrometry as previously described with minor modifications [18]. Analysis was performed using a gas chromatography system (Shimadzu Japan) coupled to a refractive index detector RID-20A (Shimadzu Japan) derivatives were separated using an Aminex HPX-87H Ion exclusion column (Bio-Rad, USA) at 40 oC for 40 min with a mobile phase of 5mM H_2SO_4 at flow rate mL/min.

For the quantification of different VFAs, external methods were employed. Standard serial solutions with various concentrations (19 mM formic acid, 17 mM acetic acid, 12 mM propionic acid, 10 mM butyric acid, 10 mM isobutyric acid, 9 mM isovaleric acid, 9 mM valeric acid) of VFAs certified composition Sigma-Aldrich, Germany were prepared [19]. The standard samples used to make the standard curve were processed in the same way as the rest of the samples. Concentrations expressed as mol/g of the VFAs were calculated using linear regression equations (R2=0.99) for the corresponding standard curves obtained using six different concentrations [20].

3. RESULTS

To investigate the pectin degradation ability of strain SF3^{T} was incubated under anaerobic conditions for 60 h in PY medium with different concentrations of pectin 0.5%,1% and 2% and one control group without the addition of any carbon sources. The result of every samples are shown in (Figs. 1). According to this data, we observed the degradation activity of strain SF3^{T} with its smoothly utilized pectin as a substrate; the highest growth ratio was observed at 2% concentration.

Table 1. The OD_{600} value, final pH and major products of strain SF3^{T} with different concentration of pectin

Substrate	Growth	OD_{600}	Final pH	VFA (mg L^{-1})		
				Acetate	Butyrate	Propionate
0.5% Pectin	+	0.095	7.5	152.134	1671.061	112.691
1% pectin	+	0.119	7.5	450.827	1779.05	211.062
2% pectin	+	1.112	7.5	187.109	1876.663	107.191

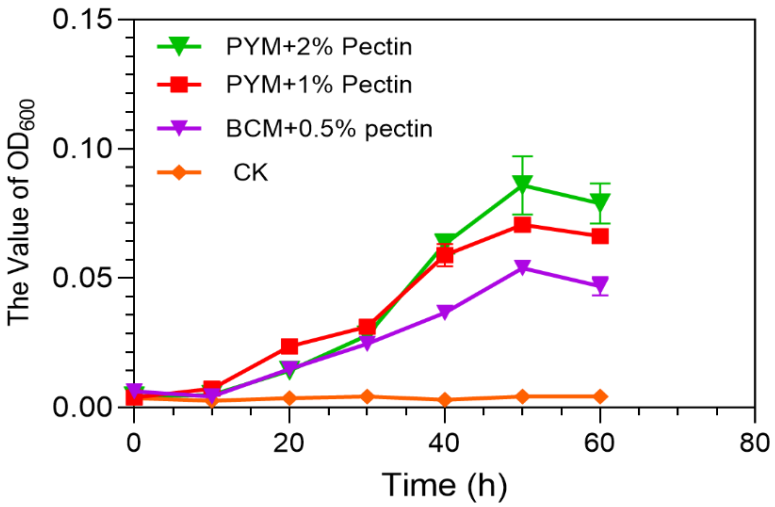

Fig. 1. The growth condition strain SF3^T in different concentrations of Pectin

3.1 Microscopic Observation

For determination of the growth condition of strain SF3^T by using the confocal fluorescent microscope (Zeiss LSM 880), cells were collected from log-phase growth conditions using DAPI dye solution we observed under blue fluorescent liens (Fig. 2. a,b and c) shown the distribution of the cells of strain SF3^T it was growing strongly in 2% concentration of pectin, according to this result and distribution of cells are increased by the addition of pectin in the PY medium [21]. The pectin is utilized as a substrate by strain SF3^{T,} the cells distribution are shown in (Fig. 2).

3.2 H_2 and CO_2 Production

The analysis of the biomass production of strain SF3^T were incubated 60 h in PY medium containing 0.5%, 1% and 2% pectin, and one negative control without any carbon sources tested three times start point, log phase, and stationary phase by (GC 2010 Shimadzu Japan) 0.5% groups can produce a trace amount of CO_2 and 1% and 2% groups are higher than the first group, but the production of H_2 was different from CO_2 0.5% did produce 1%, and 2% of pectin can produce a trace amount of H_2 the result shown in (Fig. 3 (a,b)).

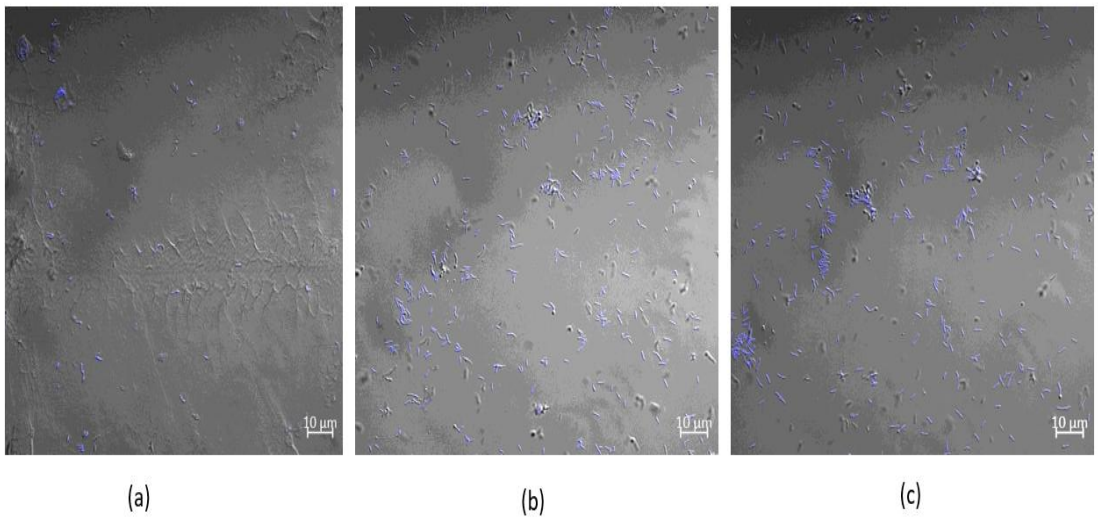

Fig. 2. (a 0.5%, b 1% and c 2% concentration of pectin) shows the cells distribution its imaged by confocal microscope

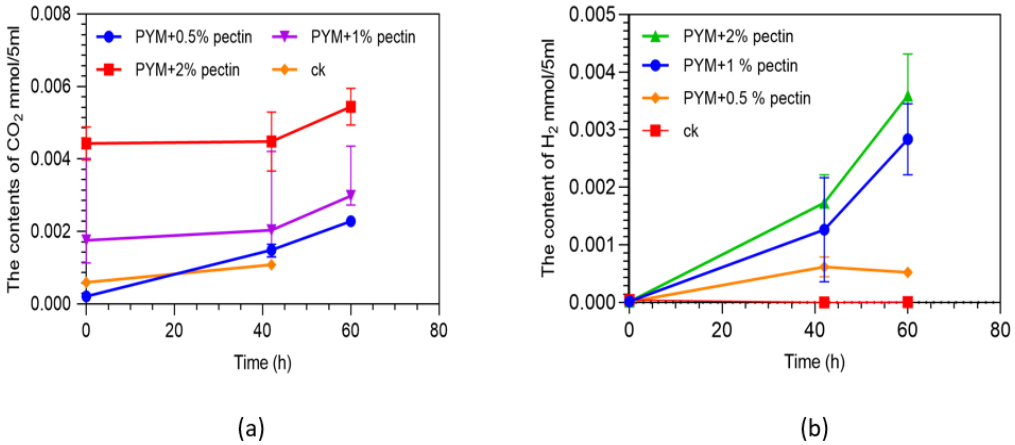

Fig. 3. Gas production of strain SF3^{I} in different concentration of Pectin

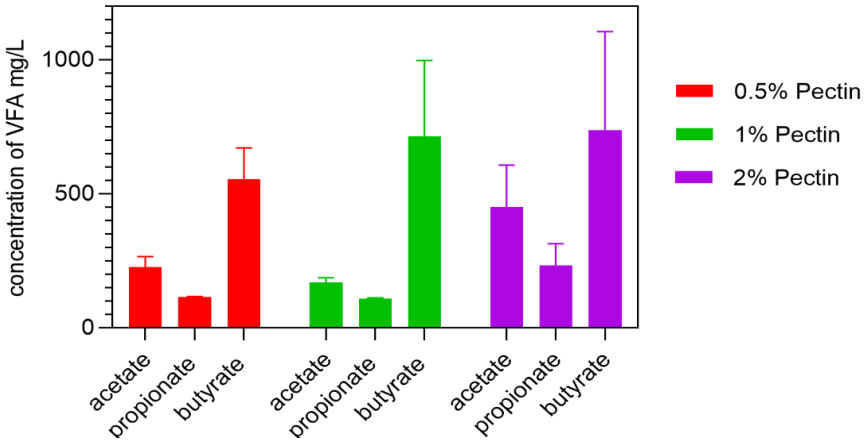

Fig. 4. Different volatile fatty acids produced by strain SF3^{T} in different concentrations of pectin

3.3 VFA Formation during Pectin and Other Substrates Fermentation

When we analyzed the VFAs of strain SF3^{T} by HPLC (Shimadzu Japan) gas chromatography system change in each samples. We observed that in inoculum with 0.5% pectin rapid increases butyrate in log phase growth condition of inoculum in pectin 1% propionate did not increase in stationary phase butyrate is increased acetate is enhanced very lite and 1%, but in 2% concentration of pectin acetate, butyrate and propionate were distinguished in stationary phase data result is shown in (Fig. 4).

4. DISCUSSION

The interaction of the gut microbiome to health and nutrition depends on its composition, which is affected by different factors, including lifestyle and diet [22]. The composition of the human gut microbiome can be changed by including non-digestible carbohydrates (prebiotics) in dietary fibber's digestion [23]. Pectin is a prebiotic dietary fiber that affects the growth condition of gut microbiome. In this study, we investigated pectin utilization by strain SF3^{T} *Hominibacterium faecale* was isolated from human feces and analyzed the ability and characteristics of strain SF3^{T} in vitro pectin fermentation through HPLC and biochemical analysis.

Pectin was mainly degraded between 20 and 45 h in all different concentrations of pectin (0.5%, 1% and 2%) during the incubation time, but the highest growth ratio was observed at 2% when pectin was digested, galacturonic acid is produced [24]. Monosaccharide such as galacturonic acid is used as an energy source by bacteria and participates in developing and

maintaining the gut microbiome [25]. In this study, the strain SF3^T showed relatively smoothly utilize the pectin as a substrate. This result suggests that acetate-producing bacteria like Hominibacterium faecale caused increased butyrate levels via butyrate synthesis using acetate as a substrate; based on this, it appears the pectin degradation results in a gut microbiota growth environment associated with the development of acetate and butyrate. In conclusion, we demonstrate that Chinese individual pectin can change the gut microbiome by measuring total sugar levels and microbial composition over time. Pectin from citrus was completely degraded by human gut microbiome strain SF3^T at 45 h *Hominibacterium faecale,* which can utilize the pectin-induced change in the gut microbiota to increase the formation of associated VFAs from 45 h on when pectin was decomposed. Pectin utilization and corresponding change to gut microbiome composition may be beneficial to human health.

5. CONCLUSION

The collective result of this study indicates that pectin degradation stimulates a wide range of microorganisms that could be directly or indirectly involved in pectin utilization or benefiting from the altered physiological conditions brought about pectin ingestions. Detailed understanding of VFAs production inside the human gut is still an unexplored area. With the advancement in omics technologies, especially metabolomics, that can be answered to expected questions.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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