Growth, metabolism, and morphology of *Akkermansia muciniphila* grown in different nutrient media

Zhi-tao Li[a], Guo-ao Hu[a], Li Zhu[b], Zheng-long Sun[c], Yun-Jiang, Min-jie Gao[a*], Xiao-bei Zhan[a*]

[a] Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education,

School of Biotechnology, Jiangnan University, Wuxi 214122, China

[b] Wuxi Galaxy Biotech Co. Ltd., Wuxi 214125, China

[c] Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences,

Suzhou 215163, China

*Corresponding authors:

Tel: +86 0510 85918299

gaominjie@msn.com (Min-jie Gao)

E-mail: xbzhan@yahoo.com (Xiao-bei Zhan)

Abstract: *Akkermansia muciniphila*, a human intestinal microbe, is a potential next-generation probiotic. Therefore, studying the in vitro cultivation of *A. muciniphila* is important. In this study, the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added human-derived (BHM) mucin media were used to ferment *A. muciniphila* in vitro. Results showed that HM had the highest biomass of *A. muciniphila* (2.89 g/L). The main metabolites of HM and PM were acetic and butyric acids, and the main metabolites of BHI medium, BPM, and BHM were acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. The butyric acid concentrations of BPM and BHM reached 9.88 and 12.88 mM, respectively. *A. muciniphila* had the highest outer membrane protein concentration in PM and HM, reaching 24.36 and 26.26 μg/mg, respectively. Electron microscopy showed that the outer membrane thickness of *A. muciniphila* was positively correlated with the outer membrane protein concentration. The appearance of *A. muciniphila* was round or elliptical in five kinds of culture media. In the BHI medium, *A. muciniphila* had the smallest diameter and length of 871 nm. This study provides a theoretical basis for the regulation of host metabolism of *A. muciniphila*.

Keywords: *Akkermansia muciniphila,* short-chain fatty acids, bioreactor, anaerobic fermentation

37 **Importance**

38 This article explains the growth, metabolism and appearance of *Akkermansia muciniphila*
39 previously described as strictly anaerobic bacteria in different nutrient media. Interestingly, the
40 nutritional composition has a presumptive relationship with *A. muciniphila* biomass, outer
41 membrane protein concentration and thickness, and diameter. At conditions containing mucin
42 as sole carbon and nitrogen sources, the metabolites of *A. muciniphila* are acetic and butyric
43 acids. This study provides a certain reference for the mechanism of action of *A. muciniphila* in
44 the host.

1. Introduction

In recent years, the research and the development of *Akkermansia muciniphila* has attracted interest[1-5]. *A. muciniphila* is an elliptical, immobile, strictly anaerobic Gram-negative strain that can use mucin in the intestine as carbon, nitrogen, and energy sources for growth[6]. The main metabolite of *A. muciniphila* is a short-chain fatty acid[7-9]. Evidence shows that *A. muciniphila* has a causal relationship with obesity[10-13], diabetes[14-16], inflammation[17-18], autism[19-20], amyotrophic lateral sclerosis[21], premature aging[22], epilepsy[23], hypertension[24], cancer[25] and metabolic abnormalities[26]. Thus, *A. muciniphila* is considered a potential probiotic especially in the prevention and the treatment of diabetes, obesity, and related metabolic disorders in terms of potential. A recent study shows that pasteurized *A. muciniphila* is better than live bacteria in preventing obesity and related complications due to the bacterial outer membrane protein Amuc-1100[13]. This discovery is important and provides an important theoretical basis for the application of the bacteria in clinical treatment. Therefore, the high-density industrial cultivation research for *A. muciniphila* is a future research hotspot.

Most intestinal bacteria are generally aerobic or facultative anaerobic bacteria. The use of nutrient-rich medium or semicombined medium with appropriate carbon source can achieve the purpose of separation and cultivation, and *A. muciniphila* is a typical strict anaerobic bacterium and extremely difficult to isolate and culture[6]. *A. muciniphila* grows slowly in a medium with glucose, N-acetylglucosamine, and N-acetylgalactosamine as carbon sources and grows well in the mucin basic medium[27-29]. The pig-derived mucin is the key component of *A. muciniphila* medium in most reports. By adding a certain amount of mucin to the brain heart

extract (BHI) broth to cultivate *A. muciniphila*, the nutritional requirements for its growth can be met. The in vitro culture of *A. muciniphila* with human mucin has not been reported yet. In porcine mucin medium, *A. muciniphila* can grow in single cells or in pairs and rarely grow in chains. *A. muciniphila* usually forms aggregates, and a translucent layer of material can be observed. In the BHI medium, this substance is rarely observed, and cells appear alone or in pairs but rarely in groups.

Previous studies show that *A. muciniphila* cultured in different media has different aggregates and cell sizes[6]. Therefore, *A. muciniphila* cultured in different media is conjectured to have evident characteristics in terms of growth, metabolites, and appearance. In this study, *A. muciniphila* was cultured in vitro by using the BHI medium and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added human-derived (BHM) mucin media. The growth status, metabolites, and appearance morphology in different media and the preliminary exploration of the mechanism of action of *A. muciniphila* and the host were determined.

2. Materials and Methods

2.1 . Strains and media

2.1.1 Strains

A. muciniphila DSM 22959 was purchased from the German Collection of Microorganisms and Cell Cultures.

2.1.2 Medium preparation

The BHI medium contained 10.0 g/L tryptone, 2.5 g/L dibasic sodium phosphate, 17.5 g/L BHI, 5.0 g/L sodium chloride, and 2.0 g/L glucose and was maintained at pH 7.4.

The PM contained 4 g/L porcine mucin, 2.5 g/L disodium hydrogen phosphate, and 5.0

g/L sodium chloride and was maintained at pH 7.4 (porcine mucin, Kuer, Beijing)

The HM contained 4 g/L human mucin, 2.5 g/L disodium hydrogen phosphate, and 5.0 g/L sodium chloride and was maintained at pH 7.4 (human mucin, extracted from pancreatic myxoma)

The BPM contained 10.0 g/L tryptone, 2.5 g/L disodium hydrogen phosphate, 17.5 g/L bovine heart dip powder, 5.0 g/L sodium chloride, 2.0 g/L glucose, and 4 g/L pig-derived mucin and was maintained at pH 7.4.

The BHM contained 10.0 g/L tryptone, 2.5 g/L disodium hydrogen phosphate, 17.5 g/L bovine heart dip powder, 5.0 g/L sodium chloride, 2.0 g/L glucose, and 4 g/L human mucin and was maintained at pH 7.4.

All media had the same concentration, magnetically stirred for 2 h, mixed well, and sterilized at 121°C for 30 min before use.

2.2. Cultivation method

2.2.1 Device introduction

An in vitro model of the bionic large intestine was developed to simulate the environment of *A. muciniphila* in the real large intestine[30]. As shown in Figure 1, the model was composed of three interconnected reaction bottles with a flexible and transparent model that simulated the internal intestinal wall and the shape of the bionic large intestine. The membrane between the reaction flask and the large intestine (Figure 1d) was filled with deionized water at 37 °C. The simulated large intestine contracted and caused peristaltic waves by controlling the pressure of the water flow of the circulating water pump through the computer. Thus, the materials in the simulated large intestine were mixed and moved in the system. This mixing was better than the

111 mixing done in the fermenter, where the phase separation of fluid and solid occurred. The
112 reaction bottle was equipped with vacuum (Figure 1g) and mixed gas (Figure 1h) control
113 devices to continuously maintain the oxygen-free environment in the simulation chamber. The
114 system was also added with a water absorption device that simulated the function of the large
115 intestine (Figure 1j). The mixture in the cavity was absorbed to simulate the formation of feces.
116 By continuously measuring the pH (Figure 11) and secreting alkaline solution (Figure 1e) to
117 neutralize the acid, the system pH was maintained to prevent the accumulation of microbial
118 metabolites (when the model accumulated, inhibition or death occurred). The system was
119 equipped with a dialysis device, which consisted of hollow fiber nanofiltration membranes that
120 filtered the metabolic products of microorganisms. Thus, the microbial metabolites in the model
121 cavity were maintained at physiological concentrations. The specific operation of dynamic
122 culture was as follows. First, the air tightness of the system was checked, and the whole reactor
123 was immersed in water. The mixed gas aerator was opened to fill the reactor cavity with gas. If
124 no bubble emerged, the air tightness of the system was good. The connection seals of each
125 reaction bottle were detected to ensure that no bubble emerged.

126 *2.2.2 Inoculation method*

127 An aliquot of frozen stock culture of *A. muciniphila* (100 μL) was inoculated in 5 mL
128 medium and incubated at 37 °C for 48 h under strict anaerobic conditions (hydrogen, 5%;
129 carbon dioxide, 10%; nitrogen, 85%). The configured medium was added into the bionic large
130 intestine in vitro model through the sample port, placed in a sterilization pot maintained at
131 121 °C for 30 min, and cooled to 37 °C. The extraction valve was opened, and the air in the
132 model was extracted. The extraction valve was closed, and the inflation valve was opened.

133 Anaerobic gas (hydrogen, 5%; carbon dioxide, 10%; nitrogen 85%) was poured into the model,
134 and the process was repeated thrice for the model to have an anaerobic environment. The seed
135 fluid flow of the incubated strain was added to the model cavity through a peristaltic pump. The
136 model peristaltic system and the dynamic cultivation of the strain were started. In addition, the
137 OD_{600} module (Figure 1m) can be added to this model to monitor the OD_{600} value of the
138 fermentation broth in real time.

139

140 Figure 1 Bionic large intestine dynamic digestion model: (A) discharge port, (B) sealing ring,
141 (C) reaction bottle, (D) simulated large intestine, (E) alkali-adding port, (F) feeding port, (G)
142 vacuum port, (H) filling mixed gas port, (I) sample port, (J) water suction device, (K) sampling
143 port, (L) pH electrode, (M) OD600 detection module, (N) peristaltic tube, (O) filter screen, (P)
144 dialysis device.

145

146 *2.2.3 . Biomass determination*

147 The fermentation broth (10 mL) was collected and centrifuged, washed twice with
148 deionized water, moved to the centrifuge tube after weighing, and centrifuged to obtain the cells.
149 The cells were dried at 105 °C and weighed.

150 *2.2.4 Determination of the short-chain fatty acids of metabolites*

151 The fermentation broth (1 mL) was collected, added with 10 μL 2-methylbutyric acid (1
152 M) as an internal standard, and slowly added with 250 μL concentrated hydrochloric acid. The
153 mixture was mixed well, added with 1 mL diethyl ether, vortexed for 1 min, and allowed to
154 stand until the organic and the water phases separated. The supernatant (organic phase) was

155 carefully collected, added with anhydrous sodium sulfate, vortexed, and passed through a 0.22
156 μm organic filter. The supernatant (5 μL) was injected into the Agilent 7890 gas chromatograph
157 equipped with an electron capture detector (Agilent, USA), which was used to determine the
158 short-chain fatty acids of *A. muciniphila*.

159 The gas chromatographic conditions were as follows: instrument, Agilent-7890A;
160 chromatography column, HP-INNOWAX; detector temperature, 250 °C; inlet temperature,
161 220 °C; flow rate, 1.5 mL/min; split ratio, 1:20; heating program, 60 °C–190 °C, 4 min; and
162 injection volume, 5 μL.

163 *2.2.5 Bacterial outer membrane protein extraction method and concentration determination*

164 *A. muciniphila* was subjected to outer membrane protein extraction using the bacterial
165 outer membrane protein extraction kit (BIOLABO HR0095, Beijing), and the protein content
166 was estimated using the enhanced BCA protein assay kit (Beyotime Biotechnology, Beijing).

167 *2.2.6 FSEM and FTEM*

168 The cultured strains were washed with PBS buffer; fixed with 2.5% glutaraldehyde
169 solution at 4 °C for 2 h; washed again with PBS buffer; dehydrated using 30%, 50%, 70%, 80%,
170 90%, and 100% alcohol; dried; sprayed with gold; and lyophilized using the SU8200 (Japan)
171 equipment. FSEM analysis was performed, and the morphology of *A. muciniphila* was observed
172 at 3.0 KV×10.0k. The cell suspension was dropped on the copper grid and then dried at room
173 temperature. TEM observations were taken by a transmission electronic microscope (JEM-I010,
174 Hitachi, Tokyo, Japan) in 120 kV.

175 *2.2.7 A. muciniphila outer membrane relative thickness and diameter measurement*

176 The TEM and SEM images were imported into the Adobe Photoshop CC 20.0.4, and the

177 ruler tool was used to measure the relative thickness and diameter of the adventitia. Each cell
178 was measured four times at different locations and averaged.

179 *2.2.8 Statistical analysis*

180 Data were expressed as mean ± SEM. Differences between the two groups were assessed
181 using the unpaired two-tailed Student t test. The data sets that involved more than two groups
182 were assessed using ANOVA. In the figures, data with * were significantly different at $P < 0.05$
183 in accordance with posthoc ANOVA. Data were analyzed using the GraphPad Prism version
184 8.00 for Windows (GraphPad Software).

185 Statistical comparisons were indicated with *, **, and *** for $P < 0.05$, $P < 0.01$, and $P <$
186 0.001, respectively.

187 **2. Results and Discussion**

188 *3.1 Effect of different media on A. muciniphila biomass*

189 First, the *A. muciniphila* biomass with different sources of mucin medium was compared.
190 As shown in Figure 2a, the *A. muciniphila* biomass grown in PM and HM slowly grew at 0–18
191 h, and the *A. muciniphila* biomass grown in PM (0.68 g/L) was higher than that in HM (0.35
192 g/L) because the mucin in the culture medium was the only carbon and nitrogen sources for the
193 growth of *A. muciniphila*. When the A. muciniphila grew, glycosidase should be secreted to
194 degrade the glycoprotein exposed to the terminal in mucin and the N-acetylgalactosamine, N-
195 acetylglucosamine, fucose, and galactose components that grow as a carbon source[31-33]. The *A.*
196 *muciniphila* in PM grew better than that in HM. This finding was because the pig-derived mucin
197 in PM was highly pure and easily degraded by *A. muciniphila*, whereas the human-derived
198 mucin in HM was extracted from the human body and only purified once*, so at the beginning,*

199 *the utilization rate of A. muciniphila to HM was not high*. The *A. muciniphila* grown in PM and
200 HM were in the logarithmic growth period from 18 h to 36 h. During this period, *A. muciniphila*
201 adapted to the growth environment of the medium, and the secretion rates of glycosidase and
202 protease accelerated, resulting in increased degradation of mucin. At 36–48 h, the biomass of
203 *A. muciniphila* in HM exceeded that in PM and reached 2.89 g/L at 48 h, and this finding may
204 be because the strain used was extracted from the human body and suitable for human mucin
205 culture medium. These results proved that the culture medium based on human mucin was
206 suitable for the growth of *A. muciniphila*. Moreover, the effects of the BHI medium, BPM, and
207 BHM on the growth performance of *A. muciniphila* were studied. As shown in Figure 2b, the
208 *A. muciniphila* grown in BPM and BHM were in logarithmic growth phase at 4–8 h, and the *A.*
209 *muciniphila* biomass in BPM (1.26 g/L) was higher than that in BHM (0.85 g/L). The *A.*
210 *muciniphila* grown in the BHI medium was in the logarithmic growth phase at 8–12 h, and the
211 *A. muciniphila* biomass of 0.61 g/L was obtained, which was lower than that in BPM and BHM.
212 The BHI, BPM and BHM subsequently entered a stable growth phase. After 48 h, the biomasses
213 in the BHI medium, BPM, and BHM were 1.17, 1.92, and 1.96 g/L, respectively. BPM and
214 BHM had better growth effects than the BHI medium because *A. muciniphila* grew best on
215 mucin-based medium. Derrien and colleagues have found that *A. muciniphila* can also grow on
216 a limited amount of sugar, including N-acetylglucosamine, N-acetylgalactosamine, and
217 glucose[6, 29]. The BHI medium contains 2 g/L glucose and has a rich nitrogen source that can
218 replace mucin. Thus, the BHI medium can support the growth of *A. muciniphila*, but the
219 biomass is only half of that obtained using the mucin medium. In addition, soy protein and
220 threonine can be used instead of mucin, and a certain amount of N-acetylglucosamine, N-

221 acetylgalactosamine, and glucose can be added to form a mixed medium to cultivate *A.*
222 *muciniphila*, and the biomass of which is not significantly different with that grown in BPM[13,
223 34]. In addition, the study found that by adding fructooligosaccharides[35-36], metformin[37-38],
224 polyphenols[39-42], probiotics[43] and fish oil unsaturated fatty acids[44] can increase the abundance
225 of *A. muciniphila* in vivo.
226
227 Figure 2 Effect of different media on the *A. muciniphila* biomass.
228 In this figure, the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added
229 porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract
230 (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added
231 human-derived (BHM) mucin media
232
233 *3.2 Effect of different media on the A. muciniphila metabolites*
234 *A. muciniphila* is an anaerobic bacterium found in the intestinal tract and a potential
235 probiotic that has a causal relationship with various chronic diseases, such as obesity and
236 diabetes. The main metabolite of *A. muciniphila* is a short-chain fatty acid. In this study, the
237 type and the concentration *of A. muciniphila* short-chain fatty acid metabolite in five kinds of
238 culture media were measured, and an interesting conclusion was obtained, as shown in Figure
239 3. The metabolites of *A. muciniphila* in HM were acetic and butyric acids in linear fatty acids,
240 whereas the metabolites of *A. muciniphila* in the BHI medium, BPM, and BHM were acetic,
241 propionic, butyric, valeric, isobutyric, and isovaleric acids in branched-chain fatty acid. In PM
242 and HM, the concentrations of acetic acid (4–6 mM) were not significantly different (Figure

243 3b), but the concentrations of butyric acid were significantly different (Figure 3a, $P < 0.05$).
244 The concentrations of butyric acid reached 1.06 mM in HM and 0.68 mM in PM. The latest
245 research shows the causal relationship between the butyric acid produced by intestinal
246 microorganisms and the risk of diabetes[45]. Butyric acid can promote the secretion of insulin by
247 β cells, regulate blood sugar, and improve the body's insulin response[46-47]. Further research is
248 needed on the mechanism of *A. muciniphila*'s main metabolites (i.e., acetic and butyric acids)
249 in PM and HM. The concentrations of acetic acid in the BHI medium, BPM, and BHM are
250 shown in Figure 3c. The concentrations of acetic acid in the BHI medium (50.43 mM) and
251 BHM (22.81 mM) were significantly different ($P < 0.05$). No significant difference was
252 observed in the concentrations of propionic acid among the BHI medium, BPM, and BHM
253 (Figure 3d). The concentrations of isobutyric acid in the BHI medium (3.15 mM) and BHM
254 (2.88 mM) were significantly different (Figure 3e, $P < 0.05$). The concentration of butyric acid
255 is shown in Fig. 3f. The concentrations of butyric acid in the BHI medium (5.79 mM), BPM
256 (9.88 mM), and BHM (12.88 mM) were significantly different ($P < 0.05$). The concentrations
257 of isovaleric acid in the BHI medium (4.25 mM) and BHM (4.07 mM) were significantly
258 different ($P < 0.05$). The concentration of valeric acid is shown in Figure 3g. The concentration
259 of valeric acid in the BHI medium was very significantly different with that in BPM ($P < 0.01$).
260 The concentrations of valeric acid in the BHI medium and BHM were very significantly
261 different ($P < 0.001$), and those of BPM and BHM were also significantly different ($P < 0.05$).
262 The valeric acid concentrations in the BHI medium, BPM, and BHM were 0.14, 0.41, and 0.51
263 mM, respectively. Studies have shown that branched-chain fatty acids, such as isobutyric and
264 isovaleric acids, are derived from the fermentation of branched-chain amino acids[48]. Compared

265 with linear short-chain fatty acids, these compounds were considered harmful to the colon and
266 metabolic health. Isobutyric and isovaleric acids were produced in the BHI medium because
267 when the carbon source in the BHI medium was exhausted or difficult to ferment, *A.*
268 *muciniphila* turned to protein fermentation, which produced toxic metabolites, such as
269 isobutyric and isovaleric acids. The contents of butyric and valeric acids in BPM and BHM
270 were relatively high, indicating that the mucin or mucin used by *A. muciniphila* was used
271 simultaneously with glucose. This specific situation needs further research.
272
273 Figure 3 Effect of different media on the metabolites of *A. muciniphila*
274 In this figure, the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added
275 porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract
276 (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added
277 human-derived (BHM) mucin media
278
279 *3.3 Effect of different media on the concentration of A. muciniphila outer membrane protein*
280 *A. muciniphila*'s outer membrane protein had a specific protein Amuc_1100, which can
281 maintain a stable state at the temperature of pasteurization and improve the intestinal barrier.
282 Through daily quantitative *A. muciniphila* feeding to mice, *A. muciniphila* can offset the
283 increase in body weight and fat caused by high-fat diet (HFD) and improve glucose tolerance
284 and insulin resistance, indicating that pasteurization *A. muciniphila* outer the beneficial effect
285 of membrane protein Amuc_1100 on HFD-induced metabolic syndrome. We quantitatively
286 detected the content of the outer membrane protein of *A. muciniphila* in five kinds of culture

287 media. As shown in Figure 4, the outer membrane protein concentrations of *A. muciniphila* in
288 the BHI medium, PM, HM, BPM, and BHM were 17.03, 24.36, 26.26, 18.34, and 19.45 μg/mg,
289 respectively. Significant differences were observed between BHI medium and BPM ($P < 0.05$),
290 BHI medium and BHM ($P < 0.05$), PM and HM ($P < 0.05$), PM and BHM ($P < 0.05$), BHI
291 medium and PM ($P < 0.01$), HM and BHM ($P < 0.01$), BHI medium and HM ($P < 0.001$), PM
292 and BPM ($P < 0.001$), and HM and BPM ($P < 0.001$). Results showed that the *A. muciniphila*
293 culture with medium containing only mucin from pig or human significantly increased the
294 concentration of the outer membrane protein. We speculated that the content of the Amuc_1100
295 protein increased to some extent. This provides a reference for the future increase in
296 Amuc_1100 A. muciniphila outer membrane protein content.
297
298 Figure 4 Effect of different media on the concentration of the *A. muciniphila* outer membrane
299 protein.
300 In this figure, the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added
301 porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract
302 (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added
303 human-derived (BHM) mucin media
304
305 *3.4 Effects of different media on the thickness and diameter of A. muciniphila outer membrane*
306 Figure 5 shows the TEM and SEM images of *A. muciniphila* in five kinds of culture media.
307 The cells of *A. muciniphila* were round or elliptical. The cells in the BHI medium grew alone
308 or in pairs, whereas the cells in PM, HM, BPM, and BHM containing mucin grew in pairs or

309 chains and even formed aggregates. The same morphology was observed with the Muct strain
310 studied by Derrien et al. As shown in Table 1, the relative thicknesses of the outer membranes
311 of *A. muciniphila* in the BHI medium, PM, HM, BPM, and BHM were 71.66, 101.20, 104.50,
312 71.46, and 72.11 nm, respectively. Significant differences were observed between BHI medium
313 and PM ($P < 0.05$), PM and BPM ($P < 0.05$), PM and BHM ($P < 0.05$), BHI medium and HM
314 ($P < 0.01$), HM and BPM ($P < 0.01$), and HM and BHM ($P < 0.01$). No significant difference
315 was observed in the other remaining groups ($P > 0.05$). The above results were the same as
316 those of the outer membrane protein concentration of *A. muciniphila*, indicating that increased
317 outer membrane thickness of the cells resulted in high outer membrane protein concentration.
318 The cell diameters of *A. muciniphila* in the BHI medium, PM, HM, BPM, and BHM were 871,
319 985, 999, 980, and 971 nm, respectively. The cell diameter in the BHI medium was significantly
320 different with those of the four other media ($P < 0.05$), and no significant difference was
321 observed between the other groups ($P > 0.05$). Derrien et al. have found that the cell size differed
322 depending on the medium. In the mucin medium, *A. muciniphila* had a diameter and length of
323 640 and 690 nm, respectively. In the BHI medium, *A. muciniphila* had a diameter and length of
324 830 nm and 1 mm, respectively, which was also different from the conclusions obtained in this
325 study. The differences were because the present study used dynamic culture, whereas the former
326 study used static culture, causing a difference in the diameter of organism. This result showed
327 that *A. muciniphila* had improved outer membrane thickness and diameter in PM and HM.
328 These advantages provide a reference for the future high-density cultivation of *A. muciniphila*.
329
330

Figure 5 SEM and TEM images *of A. muciniphila* in different media.

In the figure, a, b, c, d, f respectively BHI, PM, HM, BPM and BHM. The brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added human-derived (BHM) mucin media

Table 1 Comparative analysis of relative thickness and diameter of akk outer membrane in different culture media

Notes: a: Relative thickness of the outer membrane of *A. muciniphila,* b: Diameter of *A. muciniphila.* The brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added human-derived (BHM) mucin media

4 Conclusion

This study explored the growth status, metabolites, and appearance of *A. muciniphila* by using five different media. The biomass of *A. muciniphila* grown in HM is the highest, followed by that grown in PM, and the lowest was that grown in the BHI medium. The metabolites of *A. muciniphila* in PM and HM are acetic and butyric acids, and the main metabolites in the BHI medium, BPM and BHM are acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids. Among them, butyric and valeric acids in BPM and BHM are significantly different from those

in the BHI medium. The outer membrane protein concentrations of *A. muciniphila* in HM and PM are 35%–40% higher than that in the three other media. The relative thickness of *A. muciniphila* outer membrane is positively correlated with the concentration of the outer membrane protein. A thick outer membrane results in high outer membrane protein concentration. The appearance of *A. muciniphila* is round or oval. The diameter of *A. muciniphila* in the BHI medium is the smallest, and no significant difference in the diameter of other media is observed. This study can provide a reference for the metabolic mechanism of *A. muciniphila* to the host.

Acknowledgements

This research was supported by National Key Research and Development Program of China (2017YFD0400302), Fundamental Research Funds for Central Universities (JUSRP51504, JUSRP51632A), and Jiangsu Province Modern Agriculture Key Project (BE2018367) and the Priority Academic Program Development of Jiangsu Higher Education Institutions, the 111 Project (No. 111-2-06) is gratefully acknowledged.

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.

References

1. Cani, P. D.; de Vos, W. M., Next-Generation Beneficial Microbes: The Case of Akkermansia muciniphila. *Front Microbiol* **2017,** *8*, 1765.
2. Zhai, Q.; Feng, S.; Arjan, N.; Chen, W., A next generation probiotic, Akkermansia muciniphila. *Crit. Rev. Food Sci. Nutr.* **2019,** *59* (19), 3227-3236.
3. Zhang, T.; Li, Q.; Cheng, L.; Buch, H.; Zhang, F., Akkermansia muciniphila is a promising probiotic. *Microb Biotechnol* **2019,** *12* (6), 1109-1125.
4. van der Ark, K. C. H.; Aalvink, S.; Suarez-Diez, M.; Schaap, P. J.; de Vos, W. M.; Belzer, C., Model-driven design of a minimal medium for Akkermansia muciniphila confirms mucus adaptation. *Microb Biotechnol* **2018,** *11* (3), 476-485.
5. Zhou, K., Strategies to promote abundance of Akkermansia muciniphila, an emerging probiotics in the gut, evidence from dietary intervention studies. *J Funct Foods* **2017,** *33*, 194-201.
6. Derrien, M.; Vaughan, E. E.; Plugge, C. M.; de Vos, W. M., Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* **2004,** *54* (Pt 5), 1469-76.
7. Le Poul, E.; Loison, C.; Struyf, S.; Springael, J. Y.; Lannoy, V.; Decobecq, M. E.; Brezillon, S.; Dupriez, V.; Vassart, G.; Van Damme, J.; Parmentier, M.; Detheux, M., Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J. Biol. Chem.* **2003,** *278* (28), 25481-9.
8. Koecher, K. J.; Noack, J. A.; Timm, D. A.; Klosterbuer, A. S.; Thomas, W.; Slavin, J. L., Estimation and interpretation of fermentation in the gut: coupling results from a 24 h batch in vitro system with fecal measurements from a human intervention feeding study using fructo-oligosaccharides, inulin, gum acacia, and pea fiber. *J. Agric. Food Chem.* **2014,** *62* (6), 1332-1337.
9. Jonathan, M. C.; van den Borne, J. J. G. C.; van Wiechen, P.; Souza da Silva, C.; Schols, H. A.; Gruppen, H., In vitro fermentation of 12 dietary fibres by faecal inoculum from pigs and humans. *Food Chem.* **2012,** *133* (3), 889-897.
10. Chassaing, B.; Koren, O.; Goodrich, J. K.; Poole, A. C.; Srinivasan, S.; Ley, R. E.; Gewirtz, A. T., Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* **2015,** *519* (7541), 92-6.
11. Dao, M. C.; Everard, A.; Aron-Wisnewsky, J.; Sokolovska, N.; Prifti, E.; Verger, E. O.; Kayser, B. D.; Levenez, F.; Chilloux, J.; Hoyles, L.; Consortium, M. I.-O.; Dumas, M. E.; Rizkalla, S. W.; Dore, J.; Cani, P. D.; Clement, K., Akkermansia muciniphila and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* **2016,** *65* (3), 426-36.
12. Everard, A.; Belzer, C.; Geurts, L.; Ouwerkerk, J. P.; Druart, C.; Bindels, L. B.; Guiot, Y.; Derrien, M.; Muccioli, G. G.; Delzenne, N. M.; de Vos, W. M.; Cani, P. D., Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* **2013,** *110* (22), 9066-71.
13. Plovier, H.; Everard, A.; Druart, C.; Depommier, C.; Van Hul, M.; Geurts, L.; Chilloux, J.; Ottman, N.; Duparc, T.; Lichtenstein, L.; Myridakis, A.; Delzenne, N. M.; Klievink, J.; Bhattacharjee, A.; van der Ark, K. C.; Aalvink, S.; Martinez, L. O.; Dumas, M. E.; Maiter, D.; Loumaye, A.; Hermans, M. P.; Thissen, J. P.; Belzer, C.; de Vos, W. M.; Cani, P. D., A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat. Med.*

2017, *23* (1), 107-113.
14. Qin, J.; Li, Y.; Cai, Z.; Li, S.; Zhu, J.; Zhang, F.; Liang, S.; Zhang, W.; Guan, Y.; Shen, D.; Peng, Y.; Zhang, D.; Jie, Z.; Wu, W.; Qin, Y.; Xue, W.; Li, J.; Han, L.; Lu, D.; Wu, P.; Dai, Y.; Sun, X.; Li, Z.; Tang, A.; Zhong, S.; Li, X.; Chen, W.; Xu, R.; Wang, M.; Feng, Q.; Gong, M.; Yu, J.; Zhang, Y.; Zhang, M.; Hansen, T.; Sanchez, G.; Raes, J.; Falony, G.; Okuda, S.; Almeida, M.; LeChatelier, E.; Renault, P.; Pons, N.; Batto, J. M.; Zhang, Z.; Chen, H.; Yang, R.; Zheng, W.; Li, S.; Yang, H.; Wang, J.; Ehrlich, S. D.; Nielsen, R.; Pedersen, O.; Kristiansen, K.; Wang, J., A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **2012,** *490* (7418), 55-60.
15. Zhao, S.; Liu, W.; Wang, J.; Shi, J.; Sun, Y.; Wang, W.; Ning, G.; Liu1, R.; Hong, J., Akkermansia muciniphila improves metabolic profiles by reducing inflammation in chow diet-fed mice. *J Mol Endocrinol* **2017,** *58* (1), 1-14.
16. Depommier, C.; Everard, A.; Druart, C.; Plovier, H.; Van Hul, M.; Vieira-Silva, S.; Falony, G.; Raes, J.; Maiter, D.; Delzenne, N. M.; de Barsy, M.; Loumaye, A.; Hermans, M. P.; Thissen, J. P.; de Vos, W. M.; Cani, P. D., Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. *Nat. Med.* **2019,** *25* (7), 1096-1103.
17. Png, C. W.; Linden, S. K.; Gilshenan, K. S.; Zoetendal, E. G.; McSweeney, C. S.; Sly, L. I.; McGuckin, M. A.; Florin, T. H., Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. *Am J Gastroenterol* **2010,** *105* (11), 2420-8.
18. Zhai, R.; Xue, X.; Zhang, L.; Yang, X.; Zhao, L.; Zhang, C., Strain-Specific Anti-inflammatory Properties of Two Akkermansia muciniphila Strains on Chronic Colitis in Mice. *Front Cell Infect Microbiol* **2019,** *9*, 239.
19. Wang, L.; Christophersen, C. T.; Sorich, M. J.; Gerber, J. P.; Angley, M. T.; Conlon, M. A., Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and Bifidobacterium spp. in feces of children with autism. *Appl Environ Microbiol* **2011,** *77* (18), 6718-21.
20. Candela, M.; Rampelli, S.; Turroni, S.; Severgnini, M.; Consolandi, C.; Bellis, G. D.; Masetti, R.; Ricci, G.; Pession, A.; Brigidi, P., Unbalance of intestinal microbiota in atopic children. *BMC Microbiol.* **2012,** *12* (95), 2-9.
21. Blacher, E.; Bashiardes, S.; Shapiro, H.; Rothschild, D.; Mor, U.; Dori-Bachash, M.; Kleimeyer, C.; Moresi, C.; Harnik, Y.; Zur, M.; Zabari, M.; Brik, R. B.; Kviatcovsky, D.; Zmora, N.; Cohen, Y.; Bar, N.; Levi, I.; Amar, N.; Mehlman, T.; Brandis, A.; Biton, I.; Kuperman, Y.; Tsoory, M.; Alfahel, L.; Harmelin, A.; Schwartz, M.; Israelson, A.; Arike, L.; Johansson, M. E. V.; Hansson, G. C.; Gotkine, M.; Segal, E.; Elinav, E., Potential roles of gut microbiome and metabolites in modulating ALS in mice. *Nature* **2019,** *572* (7770), 474-480.
22. Barcena, C.; Valdes-Mas, R.; Mayoral, P.; Garabaya, C.; Durand, S.; Rodriguez, F.; Fernandez-Garcia, M. T.; Salazar, N.; Nogacka, A. M.; Garatachea, N.; Bossut, N.; Aprahamian, F.; Lucia, A.; Kroemer, G.; Freije, J. M. P.; Quiros, P. M.; Lopez-Otin, C., Healthspan and lifespan extension by fecal microbiota transplantation into progeroid mice. *Nat. Med.* **2019,** *25* (8), 1234-1242.
23. Olson, C. A.; Vuong, H. E.; Yano, J. M.; Liang, Q. Y.; Nusbaum, D. J.; Hsiao, E. Y., The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell* **2018,** *173* (7), 1728-1741 e13.
24. Li, J.; Zhao, F.; Wang, Y.; Chen, J.; Tao, J.; Tian, G.; Wu, S.; Liu, W.; Cui, Q.; Geng, B.; Zhang, W.; Weldon, R.; Auguste, K.; Yang, L.; Liu, X.; Chen, L.; Yang, X.; Zhu, B.; Cai, J., Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* **2017,** *5* (1), 14.
25. Routy, B.; Chatelier, E. L.; Derosa, L.; Duong, C. P. M.; Alou, M. T.; Daillère, R.; Fluckiger, A.; Messaoudene, M.; Rauber, C.; Roberti, M. P.; Fidelle, M., Gut microbiome influences efficacy of PD-1–

based immunotherapy against epithelial tumors. *Science* **2018,** *259*, 91-97.
26. Ijssennagger, N.; Belzer, C.; Hooiveld, G. J.; Dekker, J.; van Mil, S. W.; Muller, M.; Kleerebezem, M.; van der Meer, R., Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon. *Proc Natl Acad Sci U S A* **2015,** *112* (32), 10038-43.
27. Guo, X.; Zhang, J.; Wu, F.; Zhang, M.; Yi, M.; Peng, Y., Different subtype strains of Akkermansia muciniphila abundantly colonize in southern China. *J. Appl. Microbiol.* **2016,** *120* (2), 452-9.
28. Marcial-Coba, M. S.; Saaby, L.; Knochel, S.; Nielsen, D. S., Dark chocolate as a stable carrier of microencapsulated Akkermansia muciniphila and Lactobacillus casei. *FEMS Microbiol. Lett.* **2019,** *366* (2).
29. Derrien, M.; Collado, M. C.; Ben-Amor, K.; Salminen, S.; de Vos, W. M., The Mucin degrader Akkermansia muciniphila is an abundant resident of the human intestinal tract. *Appl Environ Microbiol* **2008,** *74* (5), 1646-8.
30. Li, Z.-t.; Zhu, L.; Zhang, W.-l.; Zhan, X.-b.; Gao, M.-j., New dynamic digestion model reactor that mimics gastrointestinal function. *Biochem. Eng. J.* **2020,** *154*.
31. Ottman, N.; Davids, M.; Suarez-Diez, M.; Boeren, S.; Schaap, P. J.; Martins Dos Santos, V. A. P.; Smidt, H.; Belzer, C.; de Vos, W. M., Genome-Scale Model and Omics Analysis of Metabolic Capacities of Akkermansia muciniphila Reveal a Preferential Mucin-Degrading Lifestyle. *Appl Environ Microbiol* **2017,** *83* (18).
32. Wang, M.; Zhang, X. Y.; Guo, R. R.; Cai, Z. P.; Hu, X. C.; Chen, H.; Wei, S.; Voglmeir, J.; Liu, L., Cloning, purification and biochemical characterization of two beta-N-acetylhexosaminidases from the mucin-degrading gut bacterium Akkermansia muciniphila. *Carbohydr Res* **2018,** *457*, 1-7.
33. Collado, M. C.; Derrien, M.; Isolauri, E.; de Vos, W. M.; Salminen, S., Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Appl Environ Microbiol* **2007,** *73* (23), 7767-70.
34. Ouwerkerk, J. P.; van der Ark, K. C. H.; Davids, M.; Claassens, N. J.; Finestra, T. R.; de Vos, W. M.; Belzer, C., Adaptation of Akkermansia muciniphila to the Oxic-Anoxic Interface of the Mucus Layer. *Appl Environ Microbiol* **2016,** *82* (23), 6983-6993.
35. Reid, D. T.; Eller, L. K.; Nettleton, J. E.; Reimer, R. A., Postnatal prebiotic fibre intake mitigates some detrimental metabolic outcomes of early overnutrition in rats. *Eur J Nutr* **2016,** *55* (8), 2399-2409.
36. Everard., A.; Lazarevic., V.; Derrien., M.; Girard., M.; Muccioli., G. G.; Neyrinck., A. M.; Possemiers., S.; Holle., A. V., Responses of Gut Microbiota and Glucose and Lipid Metabolism to Prebiotics in Genetic Obese and Diet-Induced Leptin-Resistant Mice. *Diabetes* **2011,** *60*, 2775-2786.
37. Zhou, Z. Y.; Ren, L. W.; Zhan, P.; Yang, H. Y.; Chai, D. D.; Yu, Z. W., Metformin exerts glucose-lowering action in high-fat fed mice via attenuating endotoxemia and enhancing insulin signaling. *Acta Pharmacol Sin* **2016,** *37* (8), 1063-75.
38. Lee, H.; Ko, G., Effect of metformin on metabolic improvement and gut microbiota. *Appl Environ Microbiol* **2014,** *80* (19), 5935-43.
39. Anhe, F. F.; Nachbar, R. T.; Varin, T. V.; Vilela, V.; Dudonne, S.; Pilon, G.; Fournier, M.; Lecours, M. A.; Desjardins, Y.; Roy, D.; Levy, E.; Marette, A., A polyphenol-rich cranberry extract reverses insulin resistance and hepatic steatosis independently of body weight loss. *Mol Metab* **2017,** *6* (12), 1563-1573.
40. Anhe, F. F.; Roy, D.; Pilon, G.; Dudonne, S.; Matamoros, S.; Varin, T. V.; Garofalo, C.; Moine, Q.; Desjardins, Y.; Levy, E.; Marette, A., A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. *Gut* **2015,** *64* (6), 872-83.

41. Roopchand., D. E.; Carmody., R. N.; Kuhn., P.; Moskal., K.; Rojas-Silva., P.; Turnbaugh., P. J.; Raskin, I., Dietary Polyphenols Promote Growth of the Gut Bacterium Akkermansia muciniphila and Attenuate High-Fat Diet–Induced Metabolic Syndrome. *Diabetes* **2015,** *64*, 2847-2858.

42. Anhe, F. F.; Pilon, G.; Roy, D.; Desjardins, Y.; Levy, E.; Marette, A., Triggering Akkermansia with dietary polyphenols: A new weapon to combat the metabolic syndrome? *Gut Microbes* **2016,** *7* (2), 146-53.

43. Alard, J.; Lehrter, V.; Rhimi, M.; Mangin, I.; Peucelle, V.; Abraham, A. L.; Mariadassou, M.; Maguin, E.; Waligora-Dupriet, A. J.; Pot, B.; Wolowczuk, I.; Grangette, C., Beneficial metabolic effects of selected probiotics on diet-induced obesity and insulin resistance in mice are associated with improvement of dysbiotic gut microbiota. *Environ. Microbiol.* **2016,** *18* (5), 1484-97.

44. Caesar, R.; Tremaroli, V.; Kovatcheva-Datchary, P.; Cani, P. D.; Backhed, F., Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling. *Cell Metab* **2015,** *22* (4), 658-68.

45. Sanna, S.; van Zuydam, N. R.; Mahajan, A.; Kurilshikov, A.; Vich Vila, A.; Võsa, U.; Mujagic, Z.; Masclee, A. A. M.; Jonkers, D. M. A. E.; Oosting, M.; Joosten, L. A. B.; Netea, M. G.; Franke, L.; Zhernakova, A.; Fu, J.; Wijmenga, C.; McCarthy, M. I., Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat. Genet.* **2019,** *51* (4), 600-605.

46. Belzer, C.; Chia, L. W.; Aalvink, S.; Chamlagain, B.; Piironen, V.; Knol, J.; de Vos, W. M., Microbial Metabolic Networks at the Mucus Layer Lead to Diet-Independent Butyrate and Vitamin B12 Production by Intestinal Symbionts. *mBio* **2017,** *8* (5).

47. Chia, L. W.; Hornung, B. V. H.; Aalvink, S.; Schaap, P. J.; de Vos, W. M.; Knol, J.; Belzer, C., Deciphering the trophic interaction between Akkermansia muciniphila and the butyrogenic gut commensal Anaerostipes caccae using a metatranscriptomic approach. *Antonie Van Leeuwenhoek* **2018,** *111* (6), 859-873.

48. Liebisch, G.; Ecker, J.; Roth, S.; Schweizer, S.; Ottl, V.; Schott, H. F.; Yoon, H.; Haller, D.; Holler, E.; Burkhardt, R.; Matysik, S., Quantification of Fecal Short Chain Fatty Acids by Liquid Chromatography Tandem Mass Spectrometry-Investigation of Pre-Analytic Stability. *Biomolecules* **2019,** *9* (4).

534 **List of the Tables**

535

536 Table 1 Comparative analysis of relative thickness and diameter of *A. muciniphila* outer

537 membrane in different culture media

538 Notes: a: Relative thickness of the outer membrane of *A. muciniphila,* b: Diameter of *A.*

539 *muciniphila.* The brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added

540 porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract

541 (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added

542 human-derived (BHM) mucin media.

543 **List of Figures**

544 Figure 1 Bionic large intestine dynamic digestion model: (A) discharge port, (B) sealing ring,
545 (C) reaction bottle, (D) simulated large intestine, (E) alkali-adding port, (F) feeding port, (G)
546 vacuum port, (H) filling mixed gas port, (I) sample port, (J) water suction device, (K) sampling
547 port, (L) pH electrode, (M) OD600 detection module, (N) peristaltic tube, (O) filter screen, (P)
548 dialysis device.

549

550 Figure 2 Effect of different media on the A. muciniphila biomass.
551 In this figure, the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added
552 porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract
553 (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added
554 human-derived (BHM) mucin media.

555

556 Figure 3 Effect of different media on the metabolites of A. muciniphila
557 In this figure, the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added
558 porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract
559 (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added
560 human-derived (BHM) mucin media.

561

562 Figure 4 Effect of different media on the concentration of the A. muciniphila outer membrane
563 protein.
564 In this figure, the brain heart extract (BHI) broth and porcine- (PM), human- (HM), BHI-added
565 porcine- (BPM), and BHI-added human-derived (BHM) mucin media the brain heart extract
566 (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added
567 human-derived (BHM) mucin media.

568

569 Figure 5 SEM and TEM images of A. muciniphila in different media.
570 In the figure, a, b, c, d, f respectively BHI, PM, HM, BPM and BHM. The brain heart extract
571 (BHI) broth and porcine- (PM), human- (HM), BHI-added porcine- (BPM), and BHI-added
572 human-derived (BHM) mucin media the brain heart extract (BHI) broth and porcine- (PM),
573 human- (HM), BHI-added porcine- (BPM), and BHI-added human-derived (BHM) mucin
574 media.

Figure-1

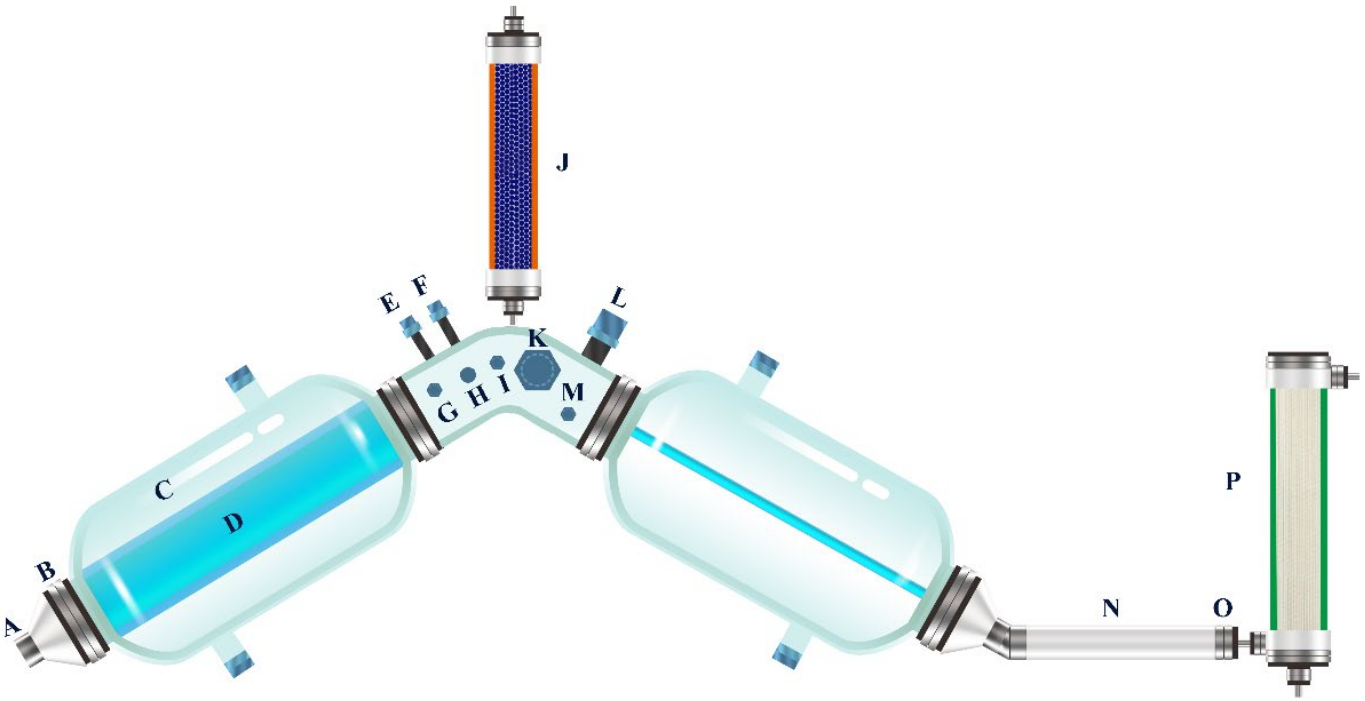


Figure-2

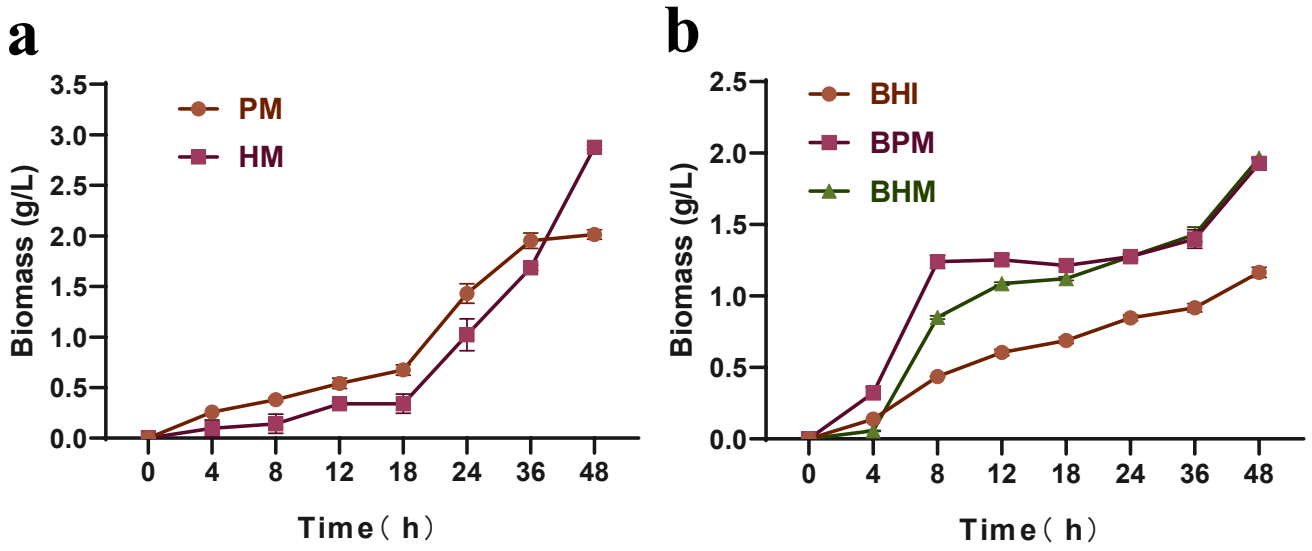

a
PM
HM
Biomass (g/L)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
4
8
12
18
24
36
48
Time（h）
b
BHI
BPM
BHM
Biomass (g/L)
2.5
2.0
1.5
1.0
0.5
0.0
0
4
8
12
18
24
36
48
Time（h）

Figure-3

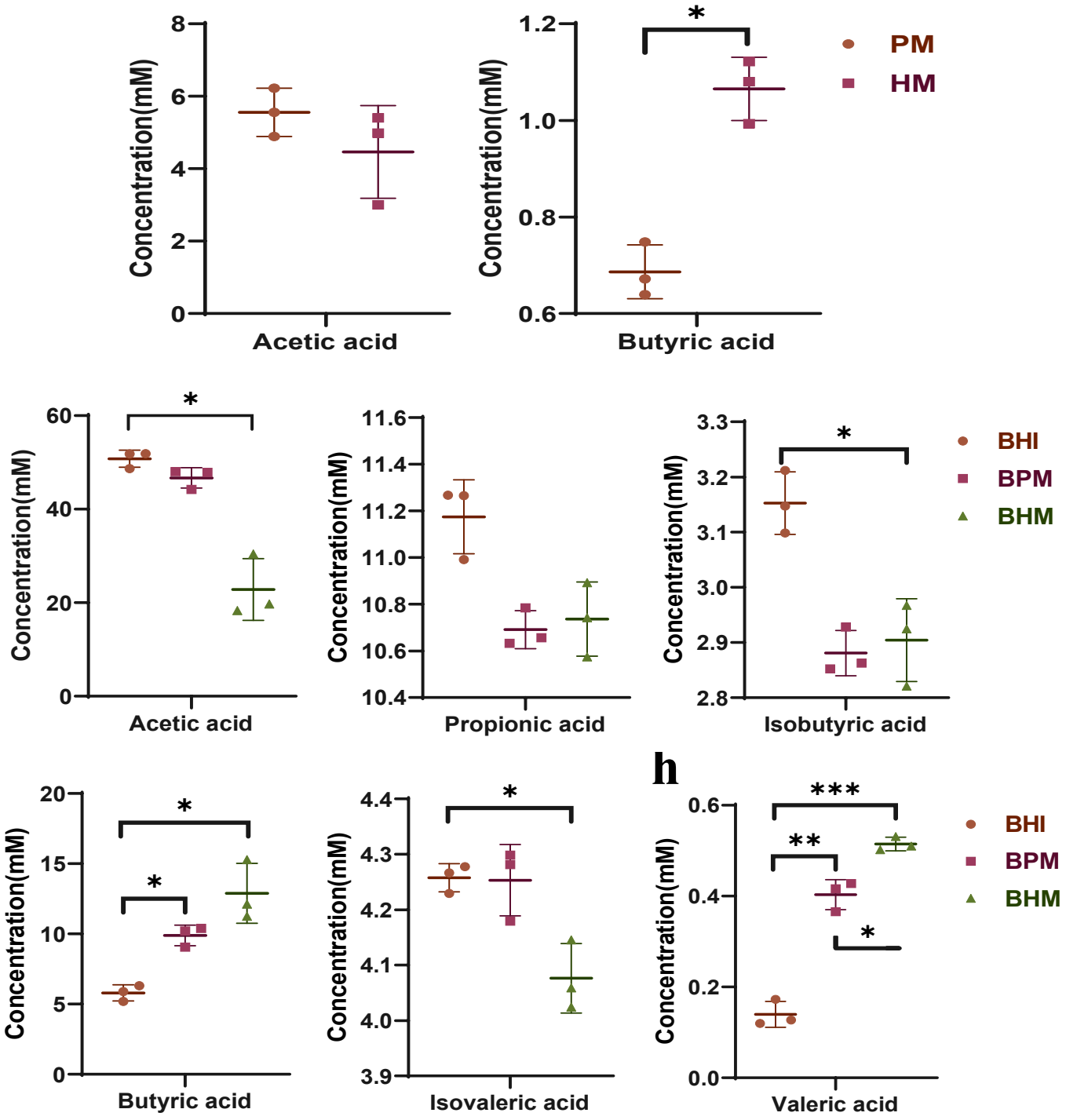

Concentration(mM)
Acetic acid
Butyric acid
PM
HM
BHI
BPM
BHM
Propionic acid
Isobutyric acid
Isovaleric acid
Valeric acid
h

Figure-4

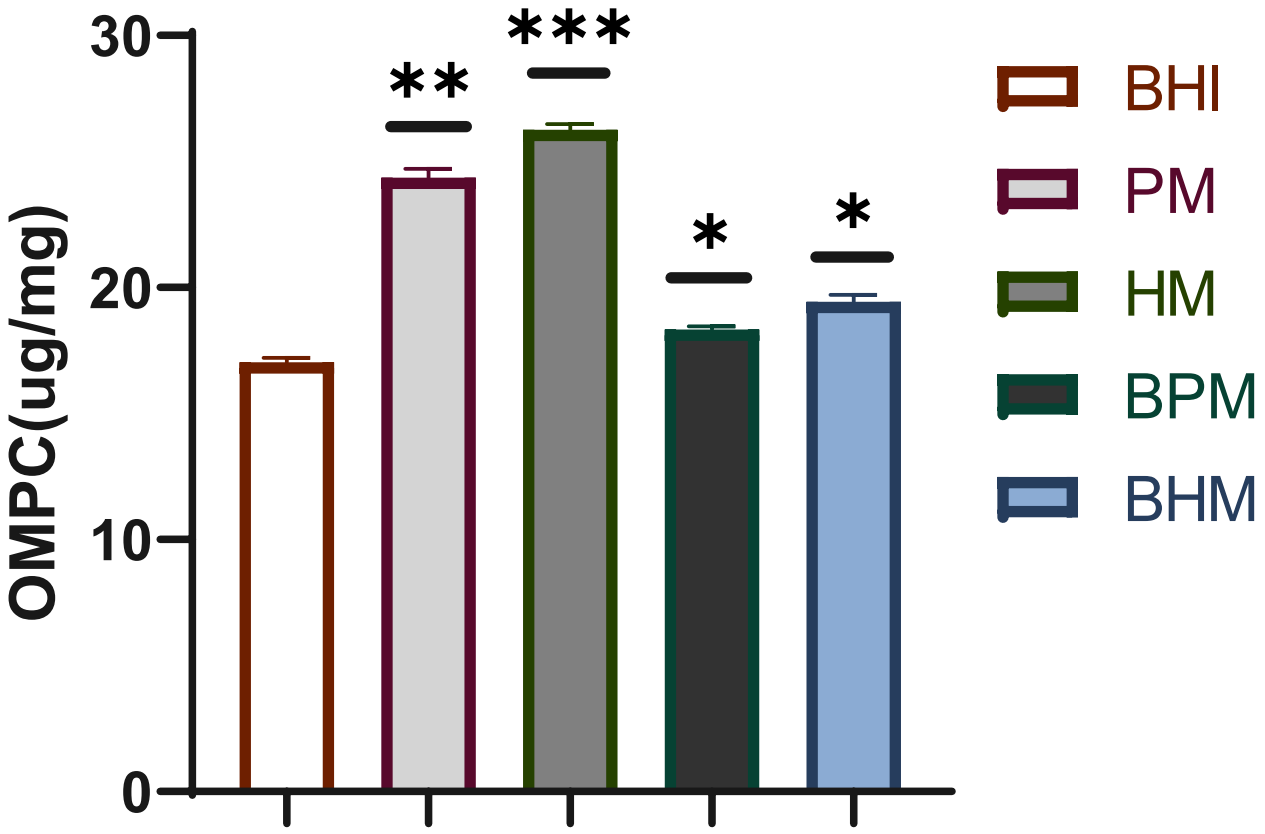
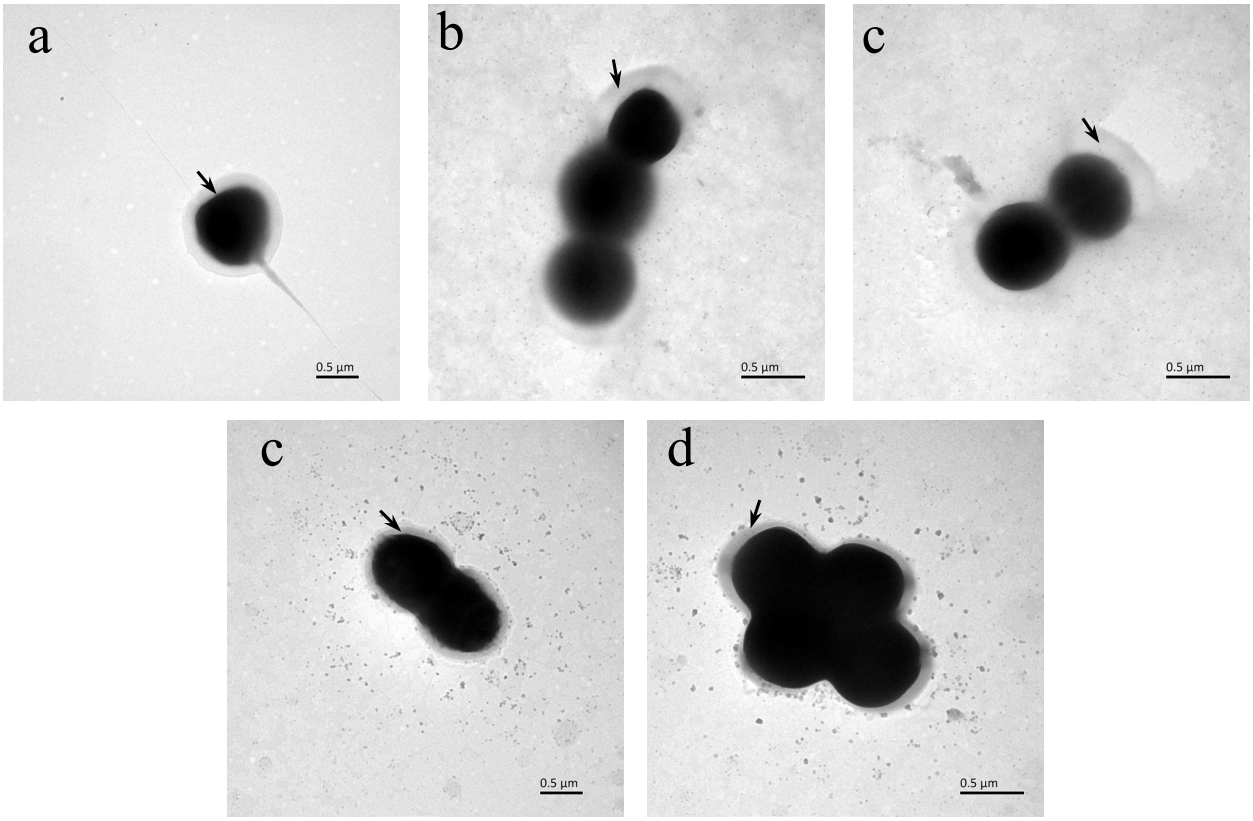
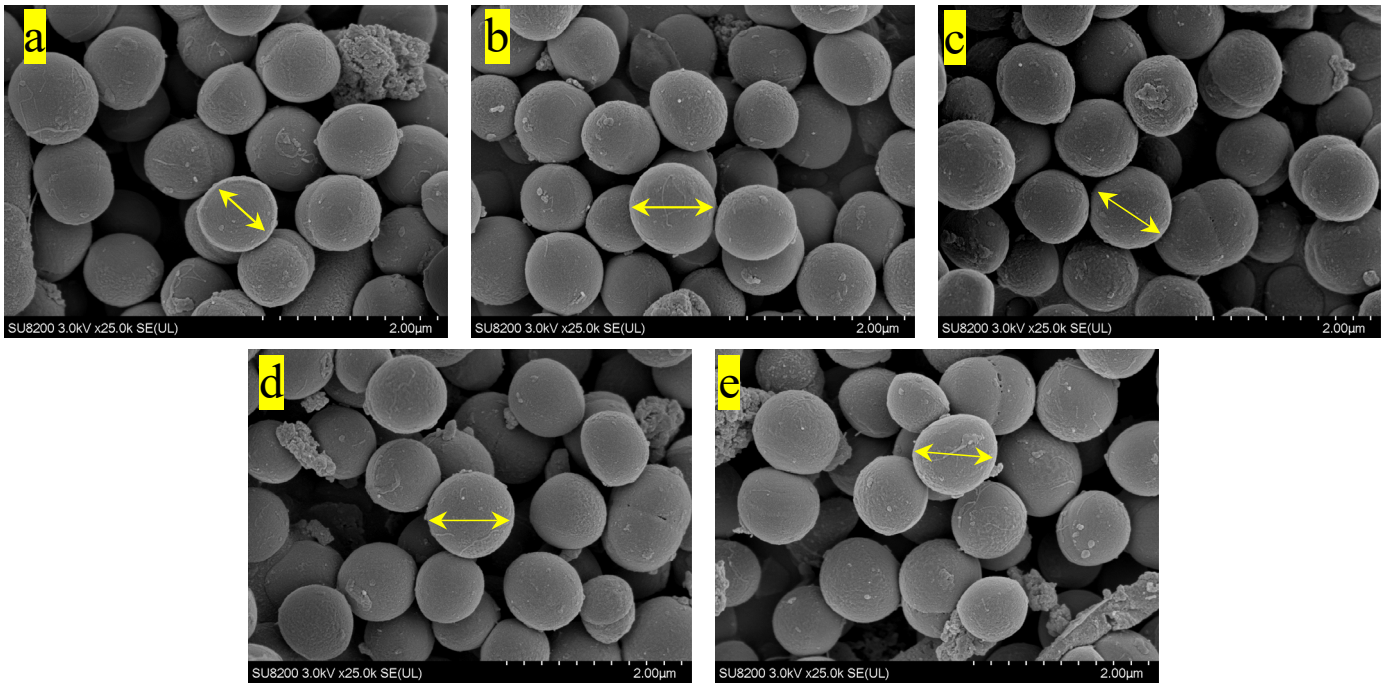


Figure-5

TEM



SEM



Table

Table 1 Comparative analysis of relative thickness and diameter of *A. muciniphila* outer *membrane in* different culture media

Tukey's multiple comparisons test	Mean 1	Mean 2	Summary	Adjusted P Value
a:BHI vs. PM	70.66	101.2	*	0.0159
a:BHI vs. HM	70.66	104.5	**	0.0072
a:BHI vs. BPM	70.66	71.46	ns	>0.9999
a:BHI vs. BHM	70.66	72.11	ns	>0.9999
a:PM vs. HM	101.2	104.5	ns	0.9934
a:PM vs. BPM	101.2	71.46	*	0.0177
a:PM vs. BHM	101.2	72.11	*	0.0152
a:HM vs. BPM	104.5	71.46	**	0.008
a:HM vs. BHM	104.5	72.11	**	0.0068
a:BPM vs. BHM	72.11	72.11	ns	>0.9999
b:BHI vs. PM	871	985	*	0.0137
b:BHI vs. PM	871	999.3	**	0.0035
b:BHI vs. BPM	871	980	*	0.0136
b:BHI vs. BHM	871	971.7	*	0.0214
b:PM vs. HM	985	999.3	ns	0.4533
b:PM vs. BPM	985	980	ns	0.9623
b:PM vs. BHM	985	971.7	ns	0.589
b:HM vs. BPM	999.3	980	ns	0.5442
b:HM vs. BHM	999.3	971.7	ns	0.3824
b:BPM vs. BHM	980	971.7	ns	0.1124