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Review Article

Syntrophic Propionate Degradation in Anaerobic Digestion: A Review

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ABSTRACT

Degradation of propionate is a central issue for improving performance of an anaerobic digestion system, because propionate is an intermediate product, which is generally accumulated in anaerobic digesters. Many factors containing pH, temperature, volatile fatty acids, hydrogen partial pressure, and toxins would inhibit the biodegradation of propionate under anaerobic conditions. Propionate can be oxidized only if a syntrophic association is carried out by propionate-oxidizing bacteria and hydrogen-consumming bacteria. As propionate degraders, syntrophic propionate-oxidizing bacteria (SPOB) play an important role in anaerobic food chain and anaerobic global carbon cycles. However, these microorganisms are often difficult to be isolated and cultured as result of extremely fastidious metabolism type. To date, only ten species were identified as SPOB, which belonged to class Deltaproteobacteria or Firmicutes. Most identified SPOB degrade propionate through methylmalonyl coenzyme A pathway (MMC) pathway and genomic information indicated the catabolic pathways are controlled by ecological factors and/or global cellular conditions. Further investigation is needed on the response mechanism of SPOB to the environmental factors. To prevent anaerobic digestor from propionate accumulation, it is important to detect SPOB in time and forecast the accumulation of propionate with mathematical models. © 2012 Friends Science Publishers

Key Words: Anaerobic digestion; Propionate degradation; Syntrophic propionate-oxidizing bacteria; Ecophysiology; Phylogeny

INTRODUCTION

Anaerobic digestion technology was paid attention by more researchers, because of the removal of organic pollutants in solid waste and wastewater and simultaneously produces methane as an energy resource (Wang *et al*., 2004; Mahmood *et al*., 2011). It is complex with a number of sequential and parallel steps that are carried out by mainly four groups of microorganisms including primary fermenting bacteria, syntrophic acetogens, homoacetogens and methanogenic archaea (Kosaka *et al*., 2006; Jha *et al*., 2011). Each group of microbes have specific metabolic functions (Fig. 1). Propionate is an important intermediate during anaerobic digestion of organic polymers. Its degradation into acetate and H_2/CO_2 (and then to CH_4) accounts for 6% ~ 35% in the total methanogenesis (Glissmann & Conrad, 2000). However, the oxidation of propionate is energetically unfavorable with a standard change in Gibbs free energy ($\Delta G^{\circ\prime}$) of +76 kJ per mol reaction as illustrated in Table I (Müller *et al*., 2010). Thermodynamically, propionate is more difficult to be anaerobically oxidized than butyrate, lactate and ethanol. So, it is usually accumulated in an anaerobic digester, even resulting in failure of the anaerobic digestion process (Kaspar & Wuhrmann, 1978; Van Lier *et al*., 1996; Shah *et al*., 2009; Ghasimi *et al*., 2009; Liu *et al*., 2010). Anaerobic oxidation of propionate is affected by many factors, including pH, temperature, hydrogen partial pressure, volatile fatty acids (VFAs), reactor configuration and organic compounds (Kim *et al*., 2002; Dhaked *et al*., 2003; Siegert & Banks, 2005; Liu *et al*., 2006).

The biodegradation of propionate depends on syntrophy between propionate-oxidizing bacteria and hydrogenotrophic methanogenic archaea in methanogenic environments (Worm *et al*., 2011). The syntrophic metabolism with other bacteria result in the pure cultivation of syntrophic propionate-oxidizing bacteria (SPOB) is very difficult. To date, only ten SPOB species have been isolated and identified (Table II). The analysis of propionate-oxidizing pathways provides information about these microorganisms in depth, including intermediates, enzymes and energy conservation. Furthermore, the genomes of several representative bacteria present the prospects to assess their unexplored functions (McInerney *et al*., 2007; Kosaka *et al*., 2008).

Propionate is always produced in anaerobic digestion and oxidized only if a syntrophic association is carried out by propionate-oxidizing bacteria and hydrogen-

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consumming bacteria (Chen *et al.,* 2005; de Bok *et al*., 2005; Ghasimi *et al*., 2009). It is accumulated in anaerobic digestion process whenever, there is a change in organic loading rate, influent pH and temperature, etc., because the syntrophic propionate-oxidizing is more difficult than other intermediate products such as butyrate and ethanol (Müller *et al*., 2010). Thus, degradation of propionate is very important for improving performance of anaerobic digestion process. However, only a few species have been obtained by now, leading to a lack in deep understanding of syntrophic propionate degradation. Based on the analysis of effect of propionate accumulation on the performance of anaerobic digestion, main factors affecting its biodegradation were discussed in this work. The currently isolated SPOB and their ecophysiological, phylogenetic and genomics were focused on as well. This summary should help to isolate more SPOB and reveal the biochemical mechanism in propionate oxidation process.

Effect of propionate accunulation on anaerobic digestion: Under high operational performance, the conversion rate is proportional to the generation rate of the intermediate products, so these compounds are hardly accumulated. However, overloading, toxicity and process parameters fluctuation disturb the process and consequently cause process instable. The process imbalance generally results in accumulation of VFAs including propionate (Pullammanppallil *et al*., 2001). The degradation of propionate into acetate is considered as one rate limiting step in anaerobic digestion system (Amani *et al*., 2011a). Furthermore, its high concentration (> 3000 mg L^{-1}) may cease the fermentation process (Boone & Xun, 1987). An increase of propionate has been observed before the failure of anaerobic digesters in treating swine, municipal sludge, and food processing water (Kaspar & Wuhrmann, 1978).

Several researchers presented that propionate accumulation affect growth, diversity and activity of the methanogens. One previous research suggested that a lot of methanogens was influenced when the propionate concentration is as low as 1500-2220 mg L^{-1} and 2 orders of magnitude of methanogens were decreased at the concentration of propionate above 5920 mg L^{-1} (Barredo & Evison, 1991). Another research also indicated that methane generation was decreased by 62~78% compared with the control at neutral pH when propionate was 5000 mg L^{-1} (Hajarnis & Ranade, 1994). The inhibition extent dramatically increased when the pH was decreased, indicating that undissociated propionate is the most toxic. In addition, Dhaked *et al*. (2003) found a two-log reduction in methanogenic counts in the slurry fermentation at pH 6.0 or 7.0 after propionate of 15000 mg L^{-1} was added into slurry. They also found that methane content of biogas decreased with the increase in propionate concentration (Dhaked *et al*., 2003).

Factors Affecting Syntrophic Propionate Degradation pH: The pH is the most important operational parameter for anaerobic digestion processes. It has direct influence on microbial growth and metabolism. Dhaked *et al*. (2003) reported that the propionate anaerobic conversion is much faster at neutral or weak alkaline pH (7 to 8) than at weak acid (pH 6). Pure culture of SPOB or co-culture of SPOB and methanogens grow in a neutral environment with the pH ranged from 6 to 8.8 (Table II). We found that propionate degradation was much faster at pH 7.0 to 8.5 than that of pH 6.5 or below; the propionate anaerobic oxidation hardly occurred at pH 5.5 (unpublished data).

Table I: Reactions involved in syntrophic propionate metabolism

Reaction	$\Delta G^{\circ'}$ (kJ mol^{-1})
Proton-reducing bacteria	
$Propionate^- + 2\ H_2O \rightarrow acetate^- + CO_2 + 3\ H_2$	+76.0
$Propionate- + 2\ H_2O + 2\ CO_2 \rightarrow Acetate- + 3\ HCOO^- + 3\ H^+$	+65.3
Methanogens	
$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$	–131.7
$4\ HCOO^- + 4\ H^+ \rightarrow CH_4 + 3\ CO_2 + 2\ H_2O$	–144.5
$Acetate^- + H+ \rightarrow CO_2 + CH_4$	–36.0

$\Delta G^{\circ'}$ (Standard Gibbs free energy change): concentration of 1 M, pH 7.0 and T = 25°C (Müller *et al.*, 2010)

Fig. 1: Anaerobic food chain of the conversion of organic matter to methane. The major microbial groups catalyzing the reactions are in oval. SPOB: Syntrophic propionate-oxidizing bacteria, SBOB: syntrophic butyrate-oxidizing bacteria (Modified from Liu and Whitman, 2008)

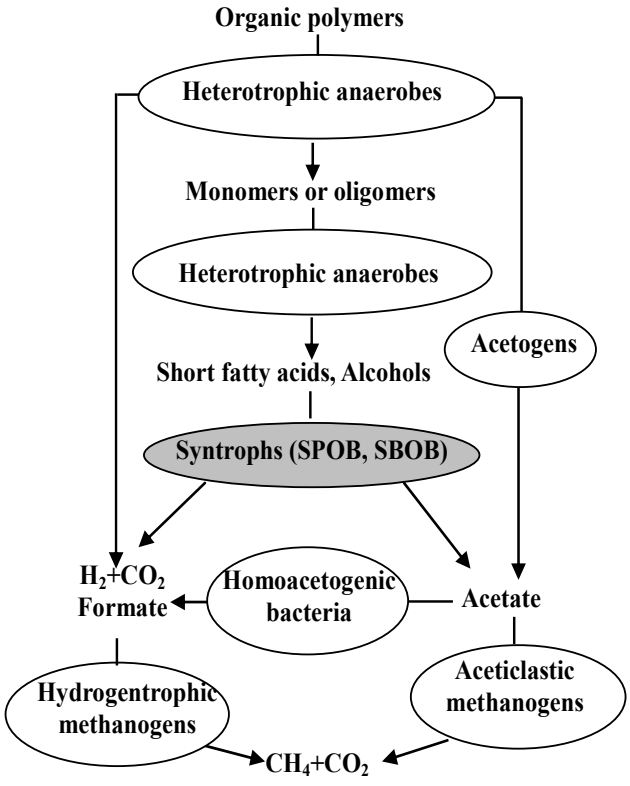


Table II: Characteristics of syntrophic propionate-oxidizing bacteria

Organism	Cell morphology	pH range	Temperature range	G+C content (mol %)	Substrates		Reference
					Pure culture	Co-culture	
Syntrophobacter wolinii	G- rod, single, in pairs or in chains, 0.6-1.0×1.0-4.5 µm, no spores, non-motile, no flagella	ND	ND	ND	Pyruvate; propionate + sulfate	Propionate	Boone & Bryant (1980); Wallrabenstein & Schink (1994)
S. pfennigii	G- slightly egg- shaped rod, single, in pairs or in chains, 1.0-1.2 × 2.2-3.0 µm, no spore, motile	6.2 - 8.0	30 - 37	ND	Propionate/Lactate+ sulfate	propionate; lactate; propanol	Wallrabenstein *et al.* (1995)
S. fumaroxidans	G-, rod or eye-shaped, single or in pairs, 1.1-1.6 × 1.8-2.5 µm, no spore, non-motile,	6.0 - 8.0	20 - 40	60.6	Propionate/H_2/formate+ fumarate; fumarate; malate; aspartate; pyruvate; propionate / formate/ succinate /H_2+ sulfate	propionate	Harmsen *et al.* (1998)
S. sulfatireducens	G-, egg-shaped, 1.0- 1.3 × 1.8-2.2 µm, single, in pairs or in chains, no spore, non-motile	6.2 - 8.8	20 – 48	58.5, 58.7	Pyruvate; propionate + sulfate/thiosulfate	propionate	Chen *et al.* (2005)
Smithella propionica	G-, slightly sinuous rod, 0.5 × 3.0-5.0 µm, weakly motile	6.3 - 7.8	23 - 40	ND	Crotonate	propionate; butyrate; malate; crotonate; fumarate	Liu *et al.* (1999)
Pelotomaculum schinkii	G^+, rod, 1.0-2.0 × 2.5 µM, spore-forming, non-motile	ND	ND	ND	None (obligately syntrophic)	propionate; fumarate + propionate	de Bok *et al.* (2005)
P. thermopropionicum	G^+, sausage-shaped rod, 0.7-0.8 × 1.7-2.8 µm, single or in pairs, spore-forming, non-motile	6.5 - 8.0	45 - 65	52.8	Pyruvate; fumarate	Propionate; ethanol; lactate; 1-butanol; 1-pentanol; 1,3-propanediol; 1-propanol; ethylene glycol	Imachi *et al.* (2002)
P. propionicum	G^+, sausage-shaped rod, 1.0 ×2.0-4.0 µm, single or in pairs, spore-forming, non-motile	6.5 - 7.5	25 - 45	ND	None (obligately syntrophic)	Propionate[a]	Imachi *et al.* (2007)
Desulfotomaculum thermocisternum	G^+, straight rod, single or in chains, 0.7-1.0× 2.0-5.2 µm, spore- forming, flagella	6.2 - 8.9	41 - 75	56	Pyruvate;H_2+CO_2/Lactate/pyruvate/propionate/ butyrate/pentanoate/hexanoate/heptanoate/ octanoate/nonanoate/decanoate/tetradecanoate/pentadecanoate/hexadecanoate/ heptadecanoate/ethanol/propanol/butanol+ sulfate	propionate	Nilsen *et al.* (1996)
D. thermobenzoicum subsp. *thermosyntrophicum*	G^+, rod with rounded ends, 1.0×3.0-11.0 µm, single or in pairs, spore-forming, slightly motile	6.0 - 8.0	45 - 62	53.7	Benzoate; fumarate; H_2+CO_2; pyruvate; lactate; propionate+ sulfate	propionate; pyruvate; lactate; fumarate; benzoate; malate; alanine; glycine	Plugge *et al.* (2002)

Temperature: Though several researchers reported that anaerobic digestion process is feasible at psychrophilic temperature, most of the reactors operate under mesophilic or thermophilic conditions (Chynoweth *et al*., 2000; Dhaked *et al*., 2003; Liu *et al*., 2006; Jha *et al*., 2011; Li *et al*., 2011). Thermodynamically, elevated temperature is beneficial to the conversion of acetate and then enhancing the degradation of propionate. It was reported that propionate was oxidized more swiftly at 55°C than that of at 38°C in single-stage batch anaerobic digestion of vegetable waste and wood chips (Hegde & Pullammanappallil, 2007). Thermophilic digestion was found to be a faster process as less butyrate and propionate were accumulated in comparison to a mesophilic process (Lu *et al*., 2007). However, Kim *et al*. (2002) found that propionic acid concentration is 31 mg L^{-1} in the effluent at mesophilic condition when the dog food as substrate uses continuous single-stage CSTR (continuous stirred-tank reactor), but 2000 mg L^{-1} at thermophilic condition.

Hydrogen partial pressure: Propionate is converted into acetate and H_2/CO_2 that are utilized by methanogens under the methanogenic environments. Several studies showed that the high hydrogen partial pressure would seriously affect the operation of anaerobic digestion systems (Boone, 1982; Harper & Pohland, 1986; Fynn & Syafila, 1990). It has been reported that the critical partial pressure for anaerobic degradation of propionate is 1×10^{-4} atm or 100 ppm (Wang *et al*., 1999; Van Lier *et al*., 1993; Zhang *et al*., 2012).

VFAs: VFAs are the important intermediates in anaerobic digestion process. Anaerobic oxidation of propionate is inhibited by VFAs and the extent of this inhibition is dependent on the VFA concentrations and pH (Siegert & Banks, 2005). These VFAs mainly contained formate, acetate, propionate and butyrate. For example, when the acetate was above 1400 mg L^{-1}, the rate of propionate

degradation was significantly decreased (Wang *et al*., 1999). It has been reported that an elevated acetate concentration had an inhibitory effect on the propionate-oxidizing bacteria (Boone & Xun, 1987; Hyun *et al*., 1998). Boone and Xun (1987) also showed that propionate degradation was severely inhibited when the 920 mg L^{-1} formate was added to medium with pH 7.2 (Boone & Xun, 1987). Besides acetate and formate, high propionate levels would inhibit the conversion of propionate. Amani *et al*. (2011b) showed the propionate had the largest inhibitory effect on the propionate removal. The propionate removal at a propionate concentration of 2986 mg L^{-1} was lower than that of 1543 mg L^{-1} by 17% at the sludge retention time for 45 h. It was observed that higher butyric acid level also inhibits the anaerobic conversion of propionate (Amani *et al*., 2011b).

Reactor configuration: Reactor configuration and the proximity between microbes play key roles in propionate anaerobic oxidation. Table III shows the difference of the propionate accumulation levels in treating dog food by different reactor configurations (Kim *et al*., 2002). In addition, propionate was competently converted into acetate in a non-mixed reactor configuration which shorted the distance of microbial consortia (Kim *et al.*, 2002).

Apart from the above factors, some other organic compounds also inhibit the propionate conversion. For example, when oleate was added at a concentration of 0.5 g L^{-1} or more, both methane production and VFAs degradation stopped immediately (Angelidaki & Ahring, 1992). Pullammanappallil *et al*. (2001) also showed that the addition of phenol caused propionate accumulation.

Charateristics of SPOB: Propionate was converted by SPOB in anaerobic conditions. SPOB are extensively present in many anaerobic ecosystems, including flooded soils, freshwater sediments, tundra, wet-wood of trees, landfills, anaerobic granular sludge and sewage digesters (Harmsen *et al*., 1996; Sekiguchi *et al*., 1999; Plugge *et al*., 2002; Lueders *et al*., 2004; McMahon *et al*., 2004). Fluorescence *in situ* hybridization reveals that a large amount of microbes, whose morphology was similar to that of *Pelotomaculum thermopropionicum*, a SPOB, are distributed in the internal layers of the thermophilic granule (Imachi *et al*., 2000). A recent study suggests that some yet uncharacterized SPOB of *Smithella syntrophus* clusters are present in the anaerobic digestor sludge (Ariesyady *et al*., 2007).

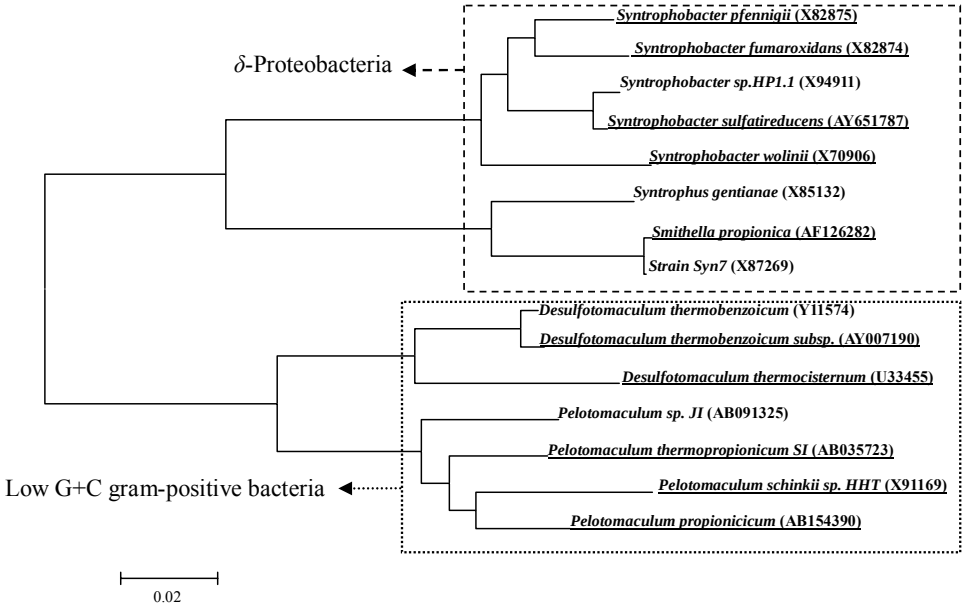
Ecophysiology: All the identified SPOB can oxidize propionate to acetate, H_2/CO_2 when they grow in co-culture with hydrogenotrophic methanogens under anaerobic conditions (Table II). In addition, *Syntrophobacter pfennigii*, *Smithella propionica*, *Pelotomaculum thermopropionicum* and *Desulfotomaculum thermobenzoicum* sub sp. *thermosyntrophicum* can also degrade some other C_3 or C_4 compounds, such as lactate, butanol, and butyrate (Wallrabenstein *et al.*, 1995; Liu *et al*., 1999, Imachi *et al*., 2002; Plugge *et al*., 2002). Besides co-culture with methanogens, most SPOB can grow on special substrates in pure culture, such as pyruvate, fumarate, and crotonate (Nilsen *et al*., 1996; Liu *et al*., 1999; Plugge *et al*., 2002; Imachi *et al*., 2002; Chen *et al*., 2005). Furthermore, the four species of *Syntrophobacter* and *Desulfotomaculum thermocisternum* have ability to axcenically grow on propionate oxidation coupling with sulfate or fumarate reduction in the deficiency of methanogens (Wallrabenstein *et al.*, 1995; Nilsen *et al*., 1996; Harmsen *et al*., 1998; Chen *et al*., 2005). However, *P. propionicum* and *P. schinkii* are two obligately syntrophic bacteria which grow only in co-culture with methanogens (de Bok *et al*., 2005; Imachi *et al*., 2007). Obligately syntrophic bacteria can not link the electrons free from propionate oxidation to fumarate reduction (de Bok *et al*., 2005). Limited capability of SPOB to convert the substrates suggests that SPOB are specific for function and environment. The fermenting microbes with higher-level dynamics are noticeably dissimilar from those of SPOB, depending on redundance to retain the general community function (Werner *et al*., 2011). This outcome suggests that biomass amendments to solve failures in propionate oxidation may be achievable in a given bioreactor, but effort to control or retain specific fermentative microbes is supposed to be very hard.

Table III: Effect of different reactor configuration on propionate conversion using dog food as feedstock in anaerobic digestion (Kim *et al.*, 2002)

Reactor Configuration	Type	Temperature (°C)	Effluent propionate level (mg L^{-1})
Single-stage CSTR	Continuously	55	2000
Single-stage CSTR	Batch-fed	55	1408
Two-phase CSTR	Batch-fed	55	1239
Single-stage CSTR	Continuously	35	31
Single-stage CSTR	Batch-fed	35	36
Two-phase CSTR	Batch-fed	35	2996

Most of the recognized SPOB degrade propionate by MMC pathway (Houwen *et al*., 1990; Plugge *et al*., 1993; Kosaka *et al*., 2006). Energetically, the main complicated step in propionate degradation is considered to be conversion of succinate to fumarate because of the necessity of the input energy is high. Genomic and biochemical analysis of *P. thermopropionicum* and *Syntrophobacter fumaroxidans* reveal the existence of succinate dehydrogenase gene cluster, indicating that a proton gradient across the membrane and energy input were needed to obtain proton from the exterior of the cytoplasmic membrane during the conversion of succinate to fumarate (Mclnerney *et al*., 2009; Stams & Plugge, 2009). *S. propionica* LYP^T had another alternative pathway, which generates acetate and butyrate through a six-carbon intermediate (de Bok *et al*., 2001). But the intermediates and enzymes related to this new pathway are not been identified. Several enzymes are involved in the MMC pathways (Kosaka *et al*., 2006). Amongst them, the fumarase is considered as the fundamental metabolic switch regulating the metabolism of matter and energy (Kosaka *et al*., 2006). The purified enzymes catalyze transformation of fumarate

Fig. 2: The evolutionary distance containing representative syntrophic propionate-oxidizing bacterial species. The neighbor-joining tree was constructed using the MEGA 3.1 software, utilizing the GenBank 16S rRNA gene database. Published syntrophic propionate-oxidizing bacteria are underlined. Accession numbers for reference sequences are shown in parentheses. The scale bar represents 2% sequence divergence. (All the 16S rRNA gene sequences from GenBank, http://www.ncbi.nlm.nih.gov)



to malate at 70°C. In addition, inactivation of fumarase in aerobic conditions is related to the transformation of the [4Fe-4S] to the inactive [3Fe-4S] form (Shimoyama, 2007).

Interspecies electron transfer is a key process for propionate degradation. In methanogenic environments, SPOB and methanogens take advantage of the metabolic abilities of their syntrophic partner to overcome energy barriers and decompose compounds that they can not be degraded by themselves. Hydrogen and formate are the primary compounds for interspecies electron transfer in methanogenic environments (Stams & Plugge, 2009). The mid-point redox potentials of the redox couples H_2/H^+ and formate/CO_2 are similar (–414 mV and –432 mV, respectively), but hydrogen and formate have different chemical and physical properties (Thauer *et al.*, 1977). The role of hydrogen and formate transfer in syntrophic degradation is still a matter of controversy. For some SPOB, hydrogen transfer may be essential for interspecies electron transfer during propionate degradation (Schmidt & Ahring, 1995). However, the formate transfer is more important than hydrogen in *S. fumaroxidans* (Dong *et al.*, 1994). In addition, genome analysis of *P. thermopropionicum* reveals the presence of multiple genes encoding formate dehydrogenase (Kosaka *et al.*, 2008). Up to now it is difficult to deduce, which one is more important in methanogenic environments. Researches with pure cultures of SPOB can shed some light on the role of hydrogen and formate in interspecies electron transfer. In particular, enzymes involved in redox reactions and the localization of electron transfer components have to be studied in more detail. It is noteworthy that direct electron transfer might also occur by so-called nanowires (Reguera *et al.*, 2005; Gorby *et al.*, 2006). Nanowires may be a novel way of interspecies electron transfer taking into account the energy metabolic characteristics of SPOB, although existence of nanowires in SPOB have not been reported.

Among the ten identified SPOB, there are seven mesophilic and three thermophilic species with culturing temperature ranges of 20 to 48°C and 41 to 75°C, respectively (Table II). Some researchers (Harmsen *et al*., 1998; Wallrabenstein *et al.*, 1995; Chen *et al*., 2005) have indicated that the favorable temperature for the mesophilic SPOB is 37°C. The favorable temperature for *P. thermopropionicum* and *D. thermobenzoicum* subsp. *thermosyntrophicum* is 55°C (Plugge *et al*., 2002; Imachi *et al*., 2002), while 62°C is recommended for *D. thermocisternum* (Nilsen *et al*., 1996).

Although all the identified SPOB exist in a neutral environment with the pH range from 6 to 8.8 (Table II), microorganisms accomplished syntrophic reaction were also present in extreme environments, including permanently cold soils, acidic soils, thermal springs, and alkaline soils (McInerney *et al*., 2009). It demonstrates that SPOB may be widely distributed at low temperature and acidophilic or basophilic environments. However, no psychrophilic or acidophilic or basophilic species have been isolated and identified. It is an opportunity and challenge to isolate SPOB from the extreme habitats in the future.

The ecophysiology of SPOB basically remained

uncharted in the both natural and artificial anaerobic digestion processes as it is exceptionally difficult to isolate and culture these microbes. The oligonucleotide probes relying on the current 16S rDNA sequences of SPOB are still limited. Thus, it is very significant to isolate and identify other SPOB from all kinds of anaerobic environments.

Phylogeny of SPOB: All currently identified SPOB are grouped into two classes, which are the class *δ*-proteobacteria in the phylum Proteobacteria and the class Clostridia within the phylum Firmicutes (Imachi *et al*., 2002; Plugge *et al*., 2002; de Bok *et al*., 2005) (Fig. 2). *Deltaproteobacteria* contained two genera *Syntrophobacter* and *Smithella*. The genus *Syntrophobacter* constituted a unique branch in the phylogenetic tree (Fig. 2). Low G+C Gram positive bacteria contain *Desulfotomaculum* and *Pelotomaculum* and these two genera belong to *Desulfotomaculum* Cluster I that is usually recognized as sulfate-reducing bacteria and have habitually been observed in different anoxic habitats (Hristova *et al*., 2000; Plugge *et al*., 2002).

Although identified SPOB are affiliated with the class *δ*-proteobacteria or the class Clostridia, recent studies show that *β*-proteobacteria are the paramount community in propionate degradation process by MAR-FISH analysis in anaerobic sludge digester (Riviere *et al*., 2009).

Genomics of SPOB: Currently, only two SPOB have been sequenced, *S. fumaroxidans* and *P. thermopropionicum* (Kosaka *et al*., 2006; McInerney *et al*., 2007). In the catabolic pathways of *P. thermopropionicum*, the propionate degradation MMC pathway forms the skeleton, which connects to some external pathways. The majority of the genes coding key catabolic enzymes are essentially associated to those for PAS domain (a signaling module) regulators. It means the catabolic pathways are controlled by ecological factors and/or global cellular conditions rather than the particular substrates (Kosaka *et al*., 2008).

However, transcription of MMC in *P. thermopropionicum* was found to be substrate-dependent (Kato *et al*., 2009). In addition, a recent study showed that the transcription levels of two formate dehydrogenase genes in *S. fumaroxidans* were higher in co-culture with methanogens than in pure culture and their transcription levels were different at different substrate, indicating that the transcription of these two formate dehydrogenase genes are dependent on growth and substrate (Worm *et al*., 2011).

CONCLUSION AND FUTURE PROPECTS

Propionate is an important intermediate product of anaerobic digestion process and its degradation is influenced by several factors including temperature, pH, reactor configuration, hydrogen partial pressure, toxins and VFAs. The accumulation of propionate has negative effects on anaerobic digestion process. As propionate degraders, SPOB occupy a unique niche in anaerobic digestion process, because of their ecological function in oxidizing propionate and then offerring substrates for methanogens. Most of the identified SPOB degrade propionate through MMC pathway and genomic information indicated the catabolic pathways are controlled by ecological factors and/or global cellular conditions.

Understanding of SPOB is limited because only ten pure cultures were obtained by now. Therefore, more SPOB should be isolated from various habitats in the future. Although it was recently suggested that direct electron transfer might also take place through so-called nanowires, the direct electron transfer between SPOB and methanogens remains to be confirmed. Ecological factors might regulate catabolic processes of SPOB, but it is not clear that how SPOB sense environmental factors to monitor cell energy levels. More research is needed on the response mechanism of SPOB to environmental factors, especially those factors regulating transcription and translation of the crucial gene in propionate degradation process. Genomics, functional genomics and culture technology are quickly rising and combining would accelerate to clarify this regulatory mechanism.

From a practical viewpoint, the quantitative and rapid detection of SPOB is essential for effective control of propionate accumulation in anaerobic digestion process. Mathematical modeling for propionate degradation in anaerobic digester should be helpful to forecast and avoid the accumulation of propionate in time.

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