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DNA microarray for detection and identification of sulfur oxidizing bacteria in Biogas Clean-up System

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Abstract

Knowledge of gene function and coexistence in the sulfur oxidizing bacteria (SOB) communities plays a significant role in driving the sulfide oxidation process in biogas clean-up process, including the stability and balance within the system. Microarray techniques can be responding to achieve as specific-detection, quantitative-identification, and high-throughput tools for microbial characterization in various environments. This research designed the microarray for monitoring SOB bacteria based on the entire genome and functional genes: *soxAXBYZ* and *fccAB*, using genome sequencing of non-SOB as a negative control, and then SOB microarray used to detect and identify SOB species-strain in the starch industry. The design of the DNA microarray revealed 61,788 probes which covered 722 strains of SOB and 35 strains of non-SOB. The quality test results demonstrated the DNA concentration, specific activity (28-36 pmol/μg), and yield (6-7 μg) of the genomic DNA had high-quality and could be repeated in the future with little noise signal. The expression level of recirculating sludge has increased in level of expression than the starting sludge. *Thiothrix*, *Syntrophomonas, Paracoccus Magnetospirillum*, *Arcobacter, Sulfuricurvum*, *Acinetobacter*, and *Hydrogenophaga* gained the gene expression level that involved with sulfide oxidation. The overexpression could be due to the biotrickling filter have more nutrients and optimal conditions for SOB growth to synthesize the necessary proteins for cell adaptation, such as SoxXA, SoxYZ, SoxB, and FccAB related to bacterial activity that oxidized the H_2S in biogas.

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Keywords: Biogas cleanup; Biogas; Biotrickling filter; Hydrogen sulfide; Sulfur oxidizing bacteria; 16S rRNA gene; Microarray; Starch industry

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Nomenclature

BPT1	Starting sludge
BPT2	Recirculating sludge
CGH	Comparative genomic hybridization
Fcc	Favocytochrome c sulfide dehydrogenase
NCBI	National center for biotechnology information
NGS	Next generation sequencing
PATRIC	Pathosystems resource integration center
SOB	Sulfur oxidizing bacteria
Sox	Sulfur oxidizing protein

1. Introduction

Thailand is the third largest producer of cassava starch in the world, with an annual yield about 31.08 million tons/year [1] from a total of 76 cassava starch industries which have the potential to produce biogas from wastewater up to 7,300,000 $m^{3/}$d or 41% of total biogas [2]. In biogas, methane and carbon dioxide are the main components. In addition, the amount of hydrogen sulfide (H_2S) is directly proportional to the protein content of biomass. Before using biogas in general, for example, for using in combustion engines, H_2S treatment must be applied to lower the H_2S concentration and do not cause corrosion problems. Usually, H_2S composition should be reduced to acceptable levels of less than 250 ppm to avoid corrosion in combustion systems, exhausts, and chimneys [3–5]. The industrial-scale of hydrogen sulfide removal system using the biological process relies on the performance of sulfur oxidizing bacteria (SOB). However, lack of a capable device to rapidly monitor the performance of SOB can cause low treatment efficiency or system failure. Presently, DNA microarray is the technology that either measures DNA or uses DNA as a part of its detection system. In the late 1990s, microscope slides made of glass and silicon began to print a known nucleotide sequence or gene is orderly arranged in specific positions with thousands of minutes spots [6]. All genomes sequence of organisms may be shown in a single slide and then using a computer database to record the pattern of fluorescence DNA sequence in each spot, they are compared to interpret the data according to what interest of things [7]. This technique enables researchers to determine the expression of thousands of microbial genes in a single reaction and take a short period to solve the problem in time [8]. Recently, a microarray technique developed for pharmacological treatment of diseases like cancer, oral precancerous lesions, genomic gains, and losses.

Besides, a microarray involved with identification of bacteria in the environment with the help of species-specific probes [7–10]. Chen et al. [8] analyzed microbial community of bioleaching system and acid mine drainage in terms of quantitation, specificity, and sensitivity from whole genomic DNA of 12 pure culture species as probes. They found that CGA array has a detection limit of approximately 0.2–5 ng with vigorous hybridization signal intensity. It achieves as a detection and identification species-specific for bacteria in acid mine drainage and bioleaching systems. Wu et al. [11] studied the potential of functional gene arrays (FGA) for assessing genes involved in nitrogen cycling: ammonia mono-oxygenase (*amoA*) genes, nitrite reductase (*nirS* and *nirK*) genes, and methane monooxygenase (*pmoA*) genes in marine sediments. The detection limit for targeted genes was approximately 25 ng of the soil community, the relative abundance of targeted genes is unclear; it needed to improve sensitivity and quantity. The critical problems of previous studies on the microarray were sensitivity and quantitative detection. However, the development of microarray technology still continues. Comparative genomic hybridization (CGH) provides better sensitivity and more accuracy for identifying the microbial species.

The aims of this research are to study the microbial communities of sulfur oxidizing bacteria obtained from a full-scale H_2S removal system by using Next Generation Sequencing (NGS) technique, and design a CGH microarray probes for detection and identification sulfur oxidizing bacteria in the starch industry.

2. Materials and methods

The experimental procedures of a DNA microarray design for detection and identification sulfur oxidizing bacteria in starch industry are shown in Fig. 1. The detail description as of followed.

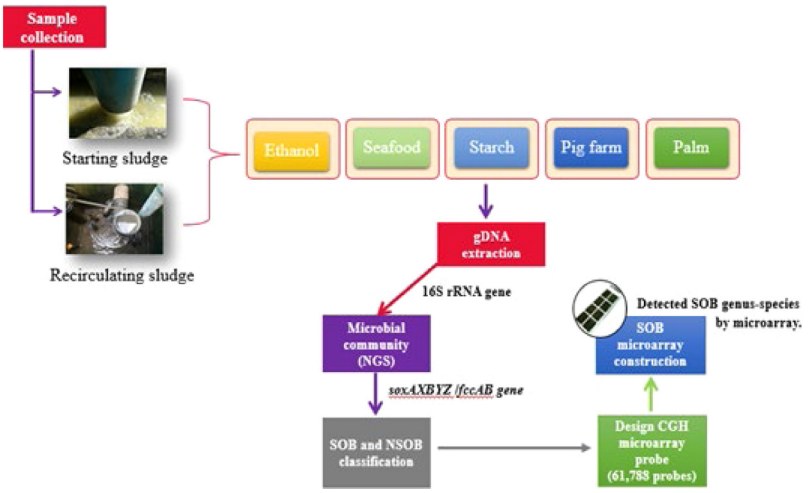

Fig. 1. Summary the experimental procedure.

2.1. Microbial sludge collection

The microbial sludge was collected from starting sludge and recirculating sludge of the full scale H_2S removal system covering all 5 industries, including cassava starch industries, palm oil mill industries, seafood industry, pig farms, and ethanol industry. After sample transportation to the laboratory, the sludge was transferred to clean tubes, and kept in a freezer at −20 °C until the genomic DNA extraction.

2.2. DNA extraction

First, the starting sludge and recirculating sludge from the full-scale biotrickling filter system were transferred to microcentrifuge tubes, and centrifugation at 12,500 rpm (MX-301, Japan) for 10 min. Next, sludge was mixed with 600 µl of the DNA extraction buffer and 600 µl of lysozyme (10 mg L^{-1}), shaking at 180 rpm at 37 °C for 1 h [12]. Then, 100 µl (20% wv^{-1}) of sodium docecil sulfate (SDS) was added and incubated at 65 °C for 30 min. After that the DNA was precipitated with isopropanol and washed with cold ethanol. Finally, the pellet was air-dried and dissolved in the elution buffer as described in Haosagul et al. [13].

2.3. Next generation sequencing (NGS)

The genomic DNA was used as a DNA template for the PCR amplification using the specific primer named 515F-806R that targeted the V4 region of the 16S rRNA gene on an Illumina HiSeq 2500 platform. According to the manufacturer's, all PCR reactions were carried out in a total 20 µl reaction: 19 µl of PCR Master Mix (1U/µl of *Taq* DNA polymerase, 0.02 mM of dNTPs, 3 mM of $MgCl_2$, 1X PCR buffers, 0.5 µM of forward and reverse primers), and 1 µl of 10 ng template DNA. *Escherichia coli* was used as the negative control in this reaction. The thermal cycle involved the initial denaturation at 95 °C (5 min) followed by denaturation at 95 °C (50 s) annealing at 55° (20 s) and elongation at 72 °C 60 (seconds). Amplification process was completed by a final extension step at 72 °C (7 min). According to the manufacturer's, the NEB Next® Ultra™ DNA Library Prep Kit was used to generate sequencing libraries for Illumina (NEB, USA)

2.4. Microarray probes design and construction

The microarray was customized with the input FASTA sequence format for tiling the sequences that consisted of "SOB" and "Non-SOB" sequences, which tiled at roughly 5,000 base pairs distance between probes. Some sequences were only designed on the genic region, which contained the *soxAXBYZ* and *fccAB* genes. FASTA files

were downloaded from the National Center for Biotechnology Information (NCBI) database, and the genes location data were collected from the Pathosystems Resource Integration Center (PATRIC) database. The microarray used in this experiment had over 60,000 spots per array. Each spot was a synthetic oligonucleotide of 60 base pairs per probe using 4 different standard phosphoramidite chemistry prints directly on the glass microarray surface with an Agilent printer. The QC process was according to the manufacturer's instructions.

2.5. Microarray processing

In brife for the summary procedure, the intact genomic DNA was labeled with Agilent SureTag Complete DNA Labeling Kit (5190-4240), according to the manufacturer instruction [14]. The labeled DNA fragments (sample and reference) were competitively hybridized onto Agilent SurePrint Custom G3 aCGH 8 × 60k Microarray (Agilent p/n G4126A_085941) for 24 h at 65 °C at 20 rpm in an Agilent hybridization oven. After hybridization, the microarray was washed in Agilent Oligo aCGH Wash buffer 1 for 5 min at room temperature and 1 min in Agilent Oligo aCGH Wash buffer 2 at 37 °C before scanning on the Agilent High-Resolution Microarray Scanner (C-model). Raw signal data were extracted from the TIFF image with the Agilent Feature Extraction for Cytogenomics version 12.1.0.3.

2.6. Microarray scanning and data analysis

The raw signal data of 61,278 probes were extracted from the scanned microarray TIFF image and translated into log ratios based on Cy3 (the recirculating sludge) over Cy5 (the starting sludge). The technical replicate features (700 probes) were combined for calculating of the standard deviation as a reproducibility check for the array data. These technical replicates were averaged before taking the average values for calculation using the log-2 ratio values between the channels. The poor-quality spots were flagged and removed from the dataset before the analysis output of the DNA copy. The region of the sequence, which was deviated between the 2 samples in a microarray, well was identified by means of the circular binary segmentation (CBS) algorithm. In this analysis, the R package DNA copy was used to run the CBS algorithm on each sample pair. The data analysis workflow was summarized in Fig. 2.

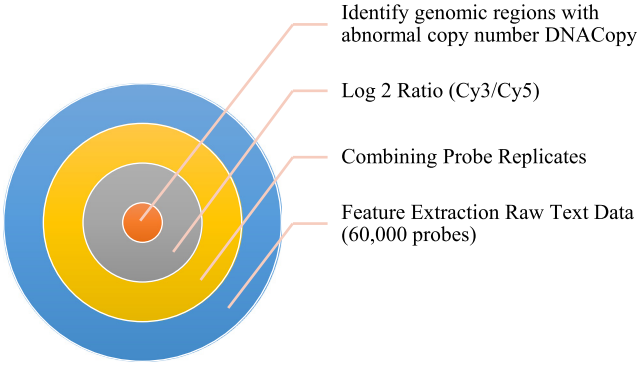

Fig. 2. Raw signal data analysis workflow.

2.7. QC metric determination

Analysis of high DNA quality samples with the Agilent's CGH microarray had QC metric determination; they could be used to assess the relative data quality and indicate the potential processing errors from the set of microarrays in an experiment. The value presented in Table 1 illustrates the criteria that the Agilent used to assess the signal quality while analyzing the samples at 3 levels: excellent, good, evaluate.

Table 1. QC metric thresholds for a CGH microarray.

Metric name	Meaning	Excellent	Good	Evaluate
BG noise	Background noise calculated as the standard deviation of negative control probes	<5	5 to 15	>15
Signal intensity	The signal intensity determined for each spot	>150	50 to 150	<50
Signal to noise	This metric calculated as the signal intensity divided by BGNoise.	>100	30 to 100	<30
Reproducibility	Replicated probes to evaluate the reproducibility of both the signals and the log ratios.	0 to 0.05	0.05 to 0.20	<0 or >0.2
DLRSD	Derivative Log2 ratio standard deviation is the standard deviation of the probe-to-probe difference of the log ratios.	<0.2	0.20 to 0.30	>0.30

3. Results and discussions

3.1. Constructed microarray sheet

The lists of the SOB and non-SOB species from NGS analysis were selected for probe designed (Fig. 3), which covers 757 species-strains (35 SOB genera and 35 genera of non-SOB). Therefore, a total of 61,788 probes (design ID: 085941) were uploaded into the Agilent SureDesign [14] for printing the microarray sheet. The characteristic of probe designed were as followed; (1) each sequence, probes captured the distance between probes, which were about 5000 bp apart, (2) each *soxAXBYZ*/*fccAB* gene region, the probes were about 400 bp apart, (3) each sequence length had 60 bases pair/probe, (4) the number of times the probe matched ≤ 3 was confirmed a good probe from the printing.

3.2. Quality of genomic DNA

According to Agilent's CGH array protocol, high-quality DNA samples have an A260/A280 ratio of $\geq$1.8, which indicates the absence of protein contaminant and A260/A230 ratio of $\geq$1.0, which indicates the absence of organic contaminants [14]. All DNA samples were tested with both Nanodrop and Qubit for further analysis. The results of the quality test with Nanodrop found that BPT2 samples had the values of A260/A280 and A260/A230 in the Agilent company criteria. In contrast, BPT1 had the value of A260/A230 lower criteria (Table 2). Subsequently, both samples were sent to Agilent to determine the DNA concentration with the Qubit fluorometer using the principles of fluorescence, which were more accurate than the measurement of the UV absorbance by Nanodrop. The results of the concentration measurement showed that the DNA concentration measured by the Qubit fluorometer decreased in both sample (Table 3), but the purity was slightly different. In particular, the BPT1 sample had the ratio of A260/A230 <1.0, indicating some of organic contaminants present in the sample or the limit of the extracted sample. However, we are continuing to test the results of A260/A230 that are less than theoretical values that may affect DNA labeling on microarray sheet (Section 3.3).

Table 2. Estimation of genomic DNA concentration by Nanodrop.

No.	Sample name	Description	A_{260}/A_{280}	A_{260}/A_{230}	NanoDrop Conc. (ng/μl)	Total amount (μg)
1	BPT1	Starting sludge-Starch	1.93	0.99	180.5	10.83
2	BPT2	Recirculating sludge-Starch	1.91	1.35	237.0	14.22

Table 3. Measure double-stranded DNA concentration before to proceed microarray.

No.	Sample name	Description	A_{260}/A_{280}	A_{260}/A_{230}	Qubit concentration	Total volume (μl)
1	BPT1	Starting sludge-Starch	1.93	0.38	154.4	40
2	BPT2	Recirculating sludge-Starch	1.96	1.27	212.0	40

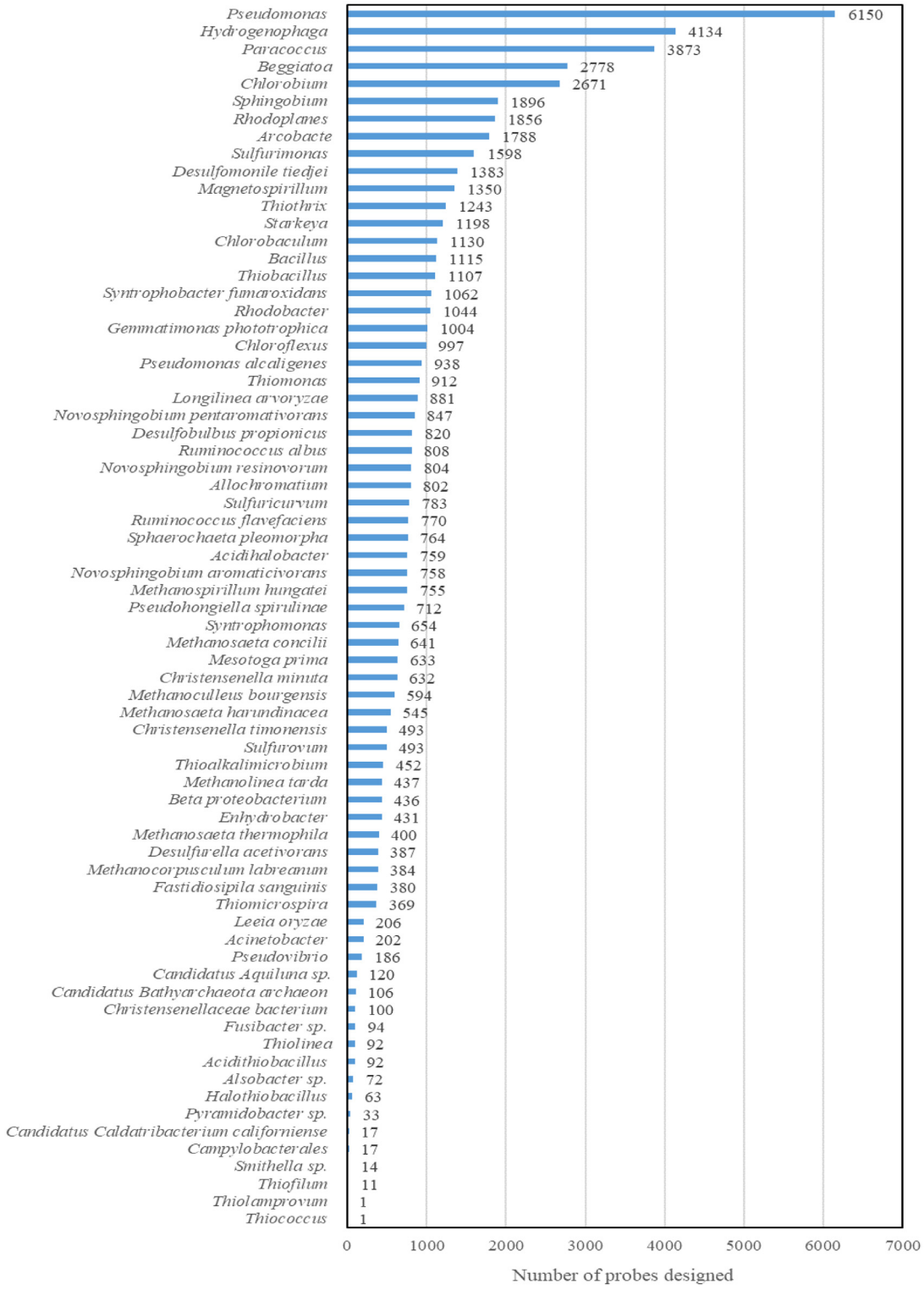

Fig. 3. The lists of the SOB and non-SOB species to design microarray probes.

3.3. Quality control of SOB microarray

The high-quality gDNA samples were isolated from the starch industries, divided into 2 groups of samples, labeled DNA with 2 fluorescent dyes (cyanine 3 and cyanine 5) and then mixed together and hybridized on the SOB microarray probes. The labeling and hybridization steps were followed the Agilent aCGH workflow. The value of yield and specific activities of each labeling reaction are shown in Table 4. The value of yield and specific activity after labeling DNA were between 6 to 7 μg and 28 to 36 pmol/μg, respectively. The quality DNA test shown that DNA concentration, specific activity, and yield of each sample were slightly differences; however, all the values were within the recommended range for further hybridization process. After the DNA labeled steps, they were co-hybridized to the arrays. The evaluation signal quality for the SOB microarray were shown in Table 5. The final quality evaluation revealed a detectable background noise in the industrial samples, however, within the acceptable evaluation range. The signal intensity from array represented an excellent value. Therefore, the signal to noise ratio was evaluated in good value. Samples demonstrated the repeatability with a little noise signal. These results indicate that BPT1 and BPT2 samples were passed QC test of hybridization process.

Table 4. The value of yield and specific activity.

Combine ID.	Sample ID.	Concentration (ng/μl)	Dye	Dye concentration (pmol/μl)	Specific activity (pmol/μg)	Yield (μg)
BPT 1&2	BPT1	691.1	Cy5	19.6	28.36	6.57
	BPT2	718.6	Cy3	25.6	35.62	6.83

Table 5. Signal quality of sample combination.

Metric name	Criteria	Value	Signal quality
BGNoise	<5	162.69	Evaluate
Signal intensity	>150	26061.90	Excellent
Signal to noise	>100	93.38	Good
Reproducibility	0 to 0.05	0.03	Excellent
DLRSD	<0.2	0.3	Good

Digital imaging systems were then applied for capture and quantify the relative fluorescence intensities of the labeled DNA probes and compared the color change between 2 sampling points (Fig. 4). The raw signal data of 61,278 probes were extracted from the scanned microarray TIFF image and translated into log-ratios based on Cy3 (the recirculating sludge) over Cy5 (the starting sludge). The unique position of the SOB strains that appear on this figure will be described in the next section.

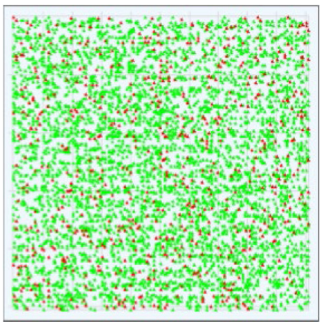

Fig. 4. Spatial distribution of the positive (green spot) and negative (red spot) log ratios (384 rows × 164 columns).. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Sulfur oxidizing bacteria detected on the microarray

The DNA microarray contained 757 specific probes that included 722 representative strains of target SOB, and 35 strains of non-SOB. Detection of each target SOB strains demonstrated that microarray can identify SOB strains with strong signal strength, and specific to the position of genes involved in the transformation of sulfides into sulfate/elemental sulfur. In this experiment, we compared the change points based on the relative intensities of each fluorophore in the log 2 ratio of the recirculating sludge (BPT2) over the starting sludge (BPT1) to identify up-regulated and down-regulated genes. *Mesotoga prima* MesG1.Ag.4.2 and *Thiothrix nivea* DSM 5205 (Fig. 5) were presented in the starch industry, and showed 20% increased from recirculating sludge over starting sludge (BPT2.BPT1). *Paracoccus* sp. displayed as the dominant genus with various strains including *Paracoccus denitrificans* strain DSM 413, *Paracoccus solventivorans* strain DSM 6637, *Paracoccus alkenifer* strain DSM 11593, *Paracoccus denitrificans* strain DSM 415, *Paracoccus pantotrophus* J46, and *Paracoccus pantotrophus* J40, with a 12%–18% of differential expression level. Moreover, the observed of differential expression of *Magnetospirillum gryphiswaldense* strain R3/S1, *Sulfuricurvum* sp. RIFCSPLOWO2, *Arcobacter cryaerophilus* strain LMG 9861, *Hydrogenophaga flava* NBRC 102514, and *Syntrophomonas zehnderi* OL-4 were similar to *Paracoccus* strains. The results suggested that most bacterial strains prefer acidic conditions in the range of pH 3–7 for adaptation and prefer an aerated condition as present in biotrickling filter tanks; the final product of the sulfide deformation is sulfate [15].

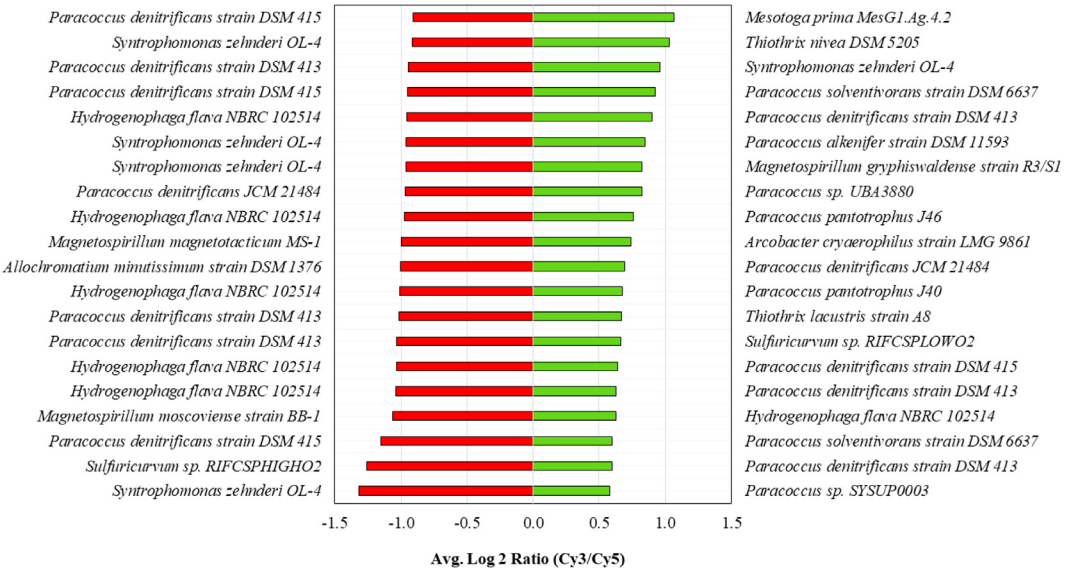

Fig. 5. The top (green bar) and bottom (red bar) members of sulfur oxidizing bacteria detected on the microarray.. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Most of these strains (Table 6) have sulfide oxidation genes: *fccAB*, which catalyzed the H_2S in vitro with the cytochrome c molecules as the electron acceptors; as such, the elemental sulfur was the final product [16]. *Mesotoga* was associated with a high COD removal efficiency and high CH_4 yield that make it as a significant member of the waste sludge [17]. In the bioreactor tanks, *Thiothrix* was usually more abundant in the outlet than in the inlet because this group preferred a lower level of H_2S/O_2 ratio and slow water flow rate [18]. The diversity of the microorganisms was also slightly different from the NGS analysis: *Sulfuricurvum* and *Thiothrix* were indicated as the top 10 members of the chemoautotrophs SOB to oxidize the H_2S in biogas while *Methanosaeta* was not found in the microarray results because it a group that grows in anaerobic conditions — it cannot be adapted and increased cell in an aeration tank like the SOB group.

The underexpressed with value of <0 was detected and might be related to a decrease in bacterial activity (cell decay) that oxidized hydrogen sulfide in biogas. The results of 20 featured species shown in Table 7. The microarray results demonstrated SOB strains within the species of *Syntrophomonas zehnderi*, *Sulfuricurvum* sp., *Paracoccus*

Table 6. Overexpression level of top 20 strains from the starch industry.

No.	Genome Name	Genome ID	Avg. Log 2 Ratio (Cy3/Cy5)	Number of Probes Supporting genomic change	Protein Expression				SOB/non-SOB
					SoxXA	SoxYZ	SoxB	FccAB	
1	*Mesotoga prima* MesG1.Ag.4.2	CP003532.1	1.0669	633	×	×	×	×	non-SOB
2	*Thiothrix nivea* DSM 5205	JH651384.1	1.0288	987	×	✓	✓	✓	SOB
3	*Syntrophomonas zehnderi* OL-4	NZ_CGIH01000048.1	0.9580	2	×	×	×	✓	SOB
4	*Paracoccus solventivorans* strain DSM 6637	FRCK01000001.1	0.9261	17	✓	✓	✓	✓	SOB
5	*Paracoccus denitrificans* strain DSM 413	NZ_FNEA01000070.1	0.9001	2	×	×	×	✓	SOB
6	*Paracoccus alkenifer* strain DSM 11593	FNXG01000003.1	0.8490	2	×	✓	×	×	SOB
7	*Magnetospirillum gryphiswaldense* strain R3/S	CP027527.1	0.8252	22	✓	✓	✓	✓	SOB
8	*Paracoccus* sp. UBA3880	DGGG01000247.1	0.8218	3	×	×	×	✓	SOB
9	*Paracoccus pantotrophus* J46	JAEM01000011.1	0.7571	2	×	✓	×	×	SOB
10	*Arcobacter cryaerophilus* strain LMG9861	NXGJ01000010.1	0.7430	4	×	×	×	✓	SOB
11	*Paracoccus denitrificans* JCM 21484	BBFH01000969.1	0.6901	2	✓	✓	✓	×	SOB
12	*Paracoccus pantotrophus* J40	JAGK01000007.1	0.6766	2	×	✓	×	×	SOB
13	*Thiothrix lacustris* strain A8	MTEJ01000720.1	0.6713	4	×	×	×	✓	SOB
14	*Sulfuricurvum* sp. RIFCSPLOWO2	MIBQ01000378.1	0.6624	2	×	×	×	✓	SOB
15	*Paracoccus denitrificans* strain DSM 415	NZ_FOYK01000060.1	0.6384	2	×	×	×	✓	SOB
16	*Paracoccus denitrificans* strain DSM 413	NZ_FNEA01000064.1	0.6293	2	×	×	×	✓	SOB
17	*Hydrogenophaga flava* NBRC 102514	NZ_BCTF01000049.1	0.6254	2	×	×	×	✓	SOB
18	*Paracoccus solventivorans* strain DSM 6637	FRCK01000007.1	0.5988	2	×	✓	×	×	SOB
19	*Paracoccus denitrificans* strain DSM 413	NZ_FNEA01000049.1	0.5971	5	×	×	×	✓	SOB
20	*Paracoccus* sp. SYSUP0003	QLUV01000001.1	0.5798	2	×	✓	×	×	SOB

Table 7. Underexpression level of bottom 20 strains from the starch industry.

No.	Genome Name	Genome ID	Avg. Log 2 Ratio (Cy3/Cy5)	Number of Probes Supporting genomic change	Protein Expression				SOB/non-SOB
					SoxXA	SoxYZ	SoxB	FccAB	
1	*Syntrophomonas zehnderi* OL-4	NZ_CGIH01000014.1	-1.3154	1	×	×	×	✓	SOB
2	*Sulfuricurvum* sp. RIFCSPHIGHO2	MIBP01000017.1	-1.2567	1	×	×	×	✓	SOB
3	*Paracoccus denitrificans* strain DSM 415	NZ_FOYK01000088.1	-1.1519	1	×	×	×	✓	SOB
4	*Magnetospirillum moscoviense* strain BB-1	LWQU01000006.1	-1.0657	1	✓	✓	×	×	SOB
5	*Hydrogenophaga flava* NBRC 102514	NZ_BCTF01000080.1	-1.0398	1	×	×	×	✓	SOB
6	*Hydrogenophaga flava* NBRC 102514	NZ_BCTF01000066.1	-1.0352	1	×	×	×	✓	SOB
7	*Paracoccus denitrificans* strain DSM 413	NZ_FNEA01000082.1	-1.0323	1	×	×	×	✓	SOB
8	*Paracoccus denitrificans* strain DSM 413	NZ_FNEA01000079.1	-1.0182	1	×	×	×	✓	SOB
9	*Hydrogenophaga flava* NBRC 102514	NZ_BCTF01000068.1	-1.0121	1	×	×	×	✓	SOB
10	*Allochromatium minutissimum* strain DSM 1376	EF618582.1	-1.0063	1	×	×	×	✓	SOB
11	*Magnetospirillum magnetotacticum* MS-1	NZ_AAAP01003032.1	-0.9951	1	✓	✓	×	×	SOB
12	*Hydrogenophaga flava* NBRC 102514	NZ_BCTF01000055.1	-0.9742	1	×	×	×	✓	SOB
13	*Paracoccus denitrificans* JCM 21484	BBFH01001039.1	-0.9677	1	✓	✓	×	×	SOB
14	*Syntrophomonas zehnderi* OL-4	NZ_CGIH01000003.1	-0.9593	1	×	×	×	✓	SOB
15	*Syntrophomonas zehnderi* OL-4	NZ_CGIH01000024.1	-0.9590	1	×	×	×	✓	SOB
16	*Hydrogenophaga flava* NBRC 102514	NZ_BCTF01000081.1	-0.9584	1	×	×	×	✓	SOB
17	*Paracoccus denitrificans* strain DSM 415	NZ_FOYK01000083.1	-0.9505	1	×	×	×	✓	SOB
18	*Paracoccus denitrificans* strain DSM 413	NZ_FNEA01000087.1	-0.9444	1	×	×	×	✓	SOB
19	*Syntrophomonas zehnderi* OL-4	NZ_CGIH01000015.1	-0.9133	1	×	×	×	✓	SOB
20	*Paracoccus denitrificans* strain DSM 415	NZ_FOYK01000077.1	-0.9109	1	×	×	×	✓	SOB

denitrificans, *Magnetospirillum moscoviense*, *Hydrogenophaga flava*, *Allochromatium minutissimum*, *Magnetospirillum magnetotacticum*, were decreased in the recirculating sludge of biotrickling filter. The underexpression of the *soxAXBYZ* genes *fccAB* specific genes was associated with sulfide oxidation pathway. In some species, decreased of differential expression could be involved with cell death in a lag phase that adapts to the sudden environmental change from anaerobic fermentation tanks into aerated tanks.

4. Conclusions

Sulfur oxidizing bacteria (SOB) plays essential role in the biogas clean-up system by removing H_2S from the biogas stream. Rare report has been done on the microbial profile analyses of SOB in the full-scale biogas clean-up system. This research was successful for detection and identification of 757 species-strains of SOB that play an important role in eliminating hydrogen sulfide in biogas. As a result of the successful CGH microarray test for

finding SOB strains in the cassava starch industry, it could be further applied to detect a wide variety of SOBs in other industries. The gene expression using a microarray has great potential in creating an in-depth understanding of the existence of sulfur oxidizing bacteria related to bacterial activity that oxidized the H_2S in biogas.

Declaration of competing interest

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

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