Identification of bacteria caused bad odors in the rubber plants

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Abstract. The problem of odor from the rubber latex industry is a major problem in the rubber industry. Due to complaints about odors from the people in the industry areas. One cause of odor may be associated with the biodegradation process by bacteria such as carbohydrates, proteins, etc., which are present in the serum. And gas generation. At present, industrial plants use water-absorbing odor to reduce the intensity of odor, but it is not very good. Therefore, if you can eliminate the microorganisms that cause odors. It may help reduce the intensity of the smell. This research was conducted to isolate microorganisms from rubber plantations and rubber plant for odor analyzed use in GC-MS technique. Comparing the gas composition with the gas data obtained from the actual air sampling. Odor composition analysis It found that the rubber and lump rubber composed with Acetic acid, propanoic acid, 2-methyl-propanoic acid, butanoic acid, 3-methyl-butanoic acid, pentanoic acid.

1. Introduction
The rubber industry is an important industry for the Thailand economy. In contrast, the amount of natural rubber production. Thailand is likely to decline. Due to weather problems in the South, rain has increased. And one of the major problems is. The bad smell of water serum affects people close to the rubber industry. For this reason, it is a major obstacle to the rubber industry in Thailand. The smell may be related to the process of biodegradation, such as carbohydrates, proteins, etc. contained in serum and gas generation. The microbes that grow in the production process are bacteria. There are three groups of bacteria is Aerobic Bacteria, Anaerobic Bacteria and Facultative Anaerobic Bacteria The smell of this acidic sour taste. According to Hoven and colleagues, volatile fatty acids is Acetic acid, Propanoic acid, Butanoic acid, 2-Methylpropanoic acid, Pentanoic acid, isovaleric acid and Hexanoic acid (Hoven et al., 2003). At present, industrial plants use water-absorbing odor to reduce the intensity of odor, but it is not very good. The composition of natural latex consists of small particles suspended in serum. The rubber is a hydrocarbon, which is polyisoprene is a part. The average molecular weight is about 106 grams per mole. (Gazeley, 1998) Rubber particles in the latex are about 30-40 percent by weight. In addition to water and rubber particles, there are also organic and inorganic minerals included. (Blackley, 1997) The composition of latex consist of. 33 percent dry...
matter, 33 percent protein, 1 percent protein, 1-2.5 percent ash, 1 percent sugar, 1 percent sugar and 1 percent water (Blackley, 1997).

2. Methods

2.1 Sampling of bacteria and air samples
The researchers collected samples of microorganisms by swab and air samples from rubber plantations and rubber plantations. Sri Trang Agro-Industry Plc. Located in Ubon Ratchathani. Take the serial dilution sample to determine the appropriate concentration of each sample so that the number of colonies in the range of 30-300 cfu/ml. Dilution at $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$. Then cultured on a Nutrient Agar Plate of 1 ml culture overnight at 37 degrees Celsius, select different colonies in each sample, then bring the Streak on the Nutrient Agar Plate overnight at room temperature. 37 degrees Celsius. Choose 1 colonies to repeat the streak plate. (Tassadaq et al., 2013) Finally a single colonized broth was fed to Nutrient Broth (NB) 3 ml for Gram straining.

2.2 PCR amplification
Pure bacteria are extracted from DNA for amplified 16s rRNA gene by Polymerase Chain Reaction. In this experiment the universal primer was used. Forward primer: 27f (5'AGAGTTTGATCMTGGCTCAG-3') Reverse primer:1492r(5'TACGGYTACCTTGTTACGACTT-3') Then analyzed the PCR product with an agarose gel electrophoresis the PCR product is purified to deliver DNA sequencing.

2.3 phylogenetic analysis
Bacterial nucleotide sequences were compared with online databases (GenBank) by alignment software (CLUSTAL W) and generated phylogenetic tree by MEGA V.7 program. The algorithm is defined as follows. Neighbour-Joining tree, Bootstrap Replications 1000, Model/Method Kimura 2-parameter model.

2.4 Preparation Selective media and Selection of Bacteria
Prepare the Selective Media, which consists of Rubber and Agar in rubber ratio: Agar is 1: 5. Then purified bacteria known to the Streak plate on Selective media incubate overnight. Transfer the colonies from each agar plate to a 1.5 ml centrifuge tube containing 1 ml of liquid containing 40% glycerol in Nutrient broth.

2.5 In vitro test generate odor from bacteria
Remove the rubber from the rubber plantation, cut into small pieces of 0.5 cm3. The rubber is put in a test tube of 3 g sterilized (sterilization is at 121 degrees Celsius 15 pounds per square inch for 15 min) Then add the above-described bacteria (500 μl volume at 8 Mcfaland) to a sterile tube, add sterile water to the test tube (control). Incubate at room temperature for 1 day, 3 days and 5 days and analysed by Sniffing.

2.6 Analysis of air samples by GC-MS technique.
Collection of air samples by comparing two methods of air sampling based on the National Institute for Occupational Safety and Health (NIOSH) and NIOSH Manual of Analytical Methods. Collect air samples in a 200 μl bag of gas and SOLID SORBENT TUBE (coconut shell charcoal, 100 mg/50 mg). That is, the adsorbent used for evaporating acid is acetic. Finally, analysed the composition of the gas by GC-MS technique.

3. Results and discussion

3.1 Identified Bacteria
The microbial isolation by doing serial dilution and then implied 16s rRNA gene fragment. Found the PCR product size analyzed by agarose gel electrophoresis approximately 1,465 base pair Figure 1.
Subsequent transmission of the nucleotide sequence by comparing the nucleotide sequence with the NCBI database. We found 39 species of bacteria.

**Figure 1.** The PCR product size approximately 1,465 base pair

**3.2 Selection of Bacteria from Selective media**

Selective media is prepared from Rubber and Agar in rubber ratio: Agar is 1: 5. The results of all 39 species tested in previous studies were isolated for the growth of good bacteria using only the nutrients contained within the latex and the rubber. It found that the number of bacteria that can grow to only 18 species in Figure 2.

![Image of bacterial cultures](image)

**Figure 2.** Bacteria gram stain; (A) Acinetobacter pittii, (B) Bacillus thuringiensis, (C) Cedecea davisa, (D) Citrobacter freundii, (E) Enterococcus faecalis, (F) Enterococcus faecium, (G) Enterobacter, (H) Klebsiella, (I) Kurthia gibsonii, (J) Lactococcus lactis, (K) Lysinibacillus xylanilyticus, (L) Myroides injenensis, (M) Pantoea wallisi, (N) Proteus mirabilis, (O) Proteus vulgaris, (P) Pseudomonas aeruginosa, (Q) Staphylococcus sciuri, and (R) Stenotrophomonas maltophilia.

**3.3 In vitro test generate odor**

This test uses a cup of rubber from the factory. Cut to 0.5 cm3, then sterilize at 121 degrees Celsius 15 pounds per square inch for 15 minutes when the sample is put into the liquid. *Bacteria* no growth was found in nutrient broth. The fluid is still clear as before the infection. While the control set is Non-sterile rubber When put in nutrient broth, it is seen that the liquid is opaque. Bacteria growing on all 18 selective media were added to sterile rubber in nutrient broth incubate of 1, 3 and 5 days. The results of the smell. Can be classified into three groups according to the foul Table 1.

**Table 1.** The odor of bacteria

| Very foul smell                        | Moderate foul smell                     | Little foul smell                         |
|---------------------------------------|----------------------------------------|------------------------------------------|
| Cedecea davisea                       | Bacillus thuringiensis                 | Acinetobacter pittii                     |
| Citrobacter freundii                  | Enterococcus faecalis                  | Myroides injenensis                      |
| Enterobacter cloacae                  | Enterococcus faecium                   | Pseudomonas aeruginosa                   |
| Klebsiella pneumonia                  | Lactococcus lactis                     | Staphylococcus sciuri                    |
| Kurthia gibsonii                      | Pantoea wallisi                        |                                          |
| Lysinibacillus xylanilyticus          |                                        |                                          |
| Proteus mirabilis                     |                                        |                                          |
Very foul smell  Moderate foul smell  Little foul smell

Proteus vulgaris  Stenotrophomonas maltophilia

3.4 GC-MS
The cups of the cups were analyzed for their aroma composition. As shown in Figure 3 six volatile fatty acids were found. Analysis of coconut shell charcoal samples was carried out by GC-MS. We found that the sample area in the production process contains acetic acid.

Figure 3. Show the chromatogram of the odor composition obtained from the analysis of rubber cups, Number 1 is acetic acid, number 2 is propanoic acid, number 3 is 2- methyl-propanoic acid, No. 4 is butanoic acid, No. 5 is 3-methyl-butanolic acid, No. 6 is pentanoic acid.

4. Conclusion
The samples were collected for selection of odor-causing bacteria in the rubber cup process. Bacteria were collected from rubber plantations and factories by selecting the bacteria from the actual sample by serial dilution and increasing the amount of genetic material to see the species. There are 39 different types of bacteria that can be isolated from selected rubber media at the ratio of latex: Agar (1:5). Can grow in food prepared from Selective media. Inspect the odor content of the rubber cup by static headspace technique by slicing the rubber cup to a small size and filling it in a glass jar and heating it to make the vapors vapor. The air gap above the sample is injected into the analyzer by the technique. GC-MS found that VFAs were composed of acetic acid, propanoic acid, butanoic acid and pentanoic acid. According to Hoven and colleagues, volatile fatty acids, such as acetic acid, propanoic acid, butanoic acid, 2-methylpropanoic acid, pentanoic acid, isovaleric acid.

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