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Evaluation of Hygienic Condition of Chinese Electronic Fly Catcher Trap Bait Using Microbiological Approach

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***Abstract*— Survival of pathogenic bacteria is usually popular topic in food studies. Thus, applying this technique to identify the hygienic condition of house flies' bait is required in order to minimize outbreaks of bait illnesses. Hence, the main objective of this paper is to study the hygienic condition of Chinese Electronic Fly Catcher Trap (CEFCT) bait which has already been proven as an effective bait for attracting flies in Malaysia. Therefore, the hygienic condition of CEFCT bait was evaluated by using the spread plate method under selective media such as de Man, Rogosa and Sharpe (MRS) agar, Eosin Methylene Blue (EMB) agar and Dichloran Rose Bengal Chlortetract Cycline (DRBC) agar. All these agars were used to identify the number of hazardous bacteria that can survive under these test conditions. A colony-forming unit (CFU) per ml was used in the experiment to estimate the number of viable plate count within the range of 1.39 to 1.56 in logarithmic scale. Eventually, experimental results show that no significant growth of E. coli, Lactobacilus yeasts and moulds were formed in four days' incubation period at 37oC. This indicates that the CEFCT bait is hygienic and can be used safely for attracting house fly without threatening wildlife and humans.**

***Keywords*— Hygienic condition; CEFCT bait; Spread plate method.**

I. INTRODUCTION

There are several kinds of baits available that are used to attract house fly in Malaysia [1]. Among them, the Chinese Electronic Fly Catcher Trap (CEFCT) bait has been proven to be the best and effective one for attracting and trapping house fly in Malaysia[2]. However, most of the baits used, pose hazard challenge to humans and the surroundings. Thus, proper hygiene of bait is needed for protection from any hazard [3]. In the others word, preventive measures should be taken to ensure that outbreaks of bait illnesses are minimized [4]. Safe foods should not contain biological and microbiological risks including physical and chemical risks. Microbiological risks define such parasite virus and pathogens bacteria which grow and develop themselves on foodstuffs that cause toxic and infection to human beings [5]. This is equally related to another study because baits were placed around the house and used frequently. Hence, due to the circumstance, it warrants the bait to get exposed to children and pets. So, using hazardous baits such as baits that contain Bacillus cereus and Escherichia coli that could be harmful to humans and animals, then makes the bait unsafe to be used [6]. Therefore, it is very important to assess the hygienic status of CEFCT bait. Eventually, microbiological analysis was conducted through hygienic bait test using Viable Plate Count in order to: identify if there is any growth of Escherichia coli, toxigenic and non-toxigenic fungi in the hygienic condition of the CEFCT; and for enrichment; cultivation; and isolation of all species of Lactobacillus [7]. Thus, it is crucial therefore to determine the hygienic condition of CEFCT baits so that the bait can be widely used by people in order to attract house fly effectively.

Microbiological hazards are considered to be a great challenge to food safety due to the potentially harmful microorganisms that have the ability to multiply rapidly from extremely small amount in food or in the human body after consumption [8]. Unfortunately, according to local news [9], most food poisoning cases in Malaysia are caused by eating food contaminated with infectious agents as the food contains harmful viruses, bacteria and parasites. Escherichia coli (E. coli), Salmonella, Shigella and Staphylococcus are the common infectious bacteria which cause food poisoning as these bacteria release poison that causes gastroenteritis, which is a major source to human diseases [10]. In addition to that, multiple surveillance on the contamination of Bacillus cereus [11] in ready-to-eat cereals, Campylobacter spp. in vegetables [12] and so on, showed the prevailing contamination of foods and equally the role of the food handler's hygiene practices in the increment or reduction of the level of contamination. As a result, preventive measures for microbial hazards need to be employed against the outbreaks of foodborne illnesses which can be applied in reinventing new house flies bait considered safe to be used.

Several kinds of methods were used in identifying microbial hazards. A variety of methods have been developed for the enumeration of microbes. These methods measure cell numbers, cell mass, or cell constituents that are proportional to the cell number [4]. The four general approaches used for estimating the sizes of microbial populations are: direct and indirect counts of

cells; and, direct and indirect measurements of microbial biomass [13]. Thus, for this study identifying microbial hazards through growing microorganism is the best way chosen. For unicellular microorganisms such as bacteria, the reproduction of the cell reproduces the entire organism [7]. Therefore, microbial growth is essentially synonymous with microbial reproduction [10]. To determine rates of microbial growth and death, it is necessary to enumerate microorganisms [14], that is to determine the bacteria numbers [15]. It is also often essential to determine the number of microorganisms in a given sample. For example, [3] stated that the ability to determine the safety of bait and food depends on knowing the level of microorganisms in those products.

II. The Material and Method

In this study, the researchers conducted an experiment for the enumeration on CEFCT baits. Spread plate method was chosen in order to identify the microbial hazard in CEFCT bait. All the procedures for the experiments were thus described in this part. For the procedure, colony-forming unit (CFU) was identified by using bacteria plate count method as described. Furthermore, the researchers went ahead to also describe the growth requirement for the enumeration process and the methods for counting the bacteria. Subsequently, the description about composition, uses and colony characteristics on the selected agar were detailed.

A. Enumeration Process for CEFCT Bait

A total of eight samples of Chinese Electronic Fly Catcher (CEFCT) baits combined with molasses were prepared at eight different times presented as A-30minutes, B-2hours, C-4hours, D-6hours, E-8hours, F-12hours, G-Control CEFCT Bait and H-Control Molasses. Each sample was followed by labelling the saline tube of the CEFCT bait for the eight different samples (A-30minutes, B-2hours, C-4hours, D-6hours, E-8hours, F-12hours, control CEFCT powder and control molasses).

More so, sample preparation for hygienic study was conducted on three types of media as shown in figure 1. The media presented were de Man, Rogosa and Sharpe (MRS), Eosin Methylene Blue Agar (EMB), and Dichloran Rose Bengal Chlortetract Cycline (DRBC).

Fig. 1 Images of selected agars: (A) MRS Agar, (B) EMB Agar, (C) DRBC Agar

Using a new sterile pipette, aseptically transferred 1.0 ml from sample tube and plate to petri dish. As shown in figure 1, the three types of media used were labelled 1 (MRS), 2 (EMB), and 3 (DRBC). This step was repeated for all the eight samples used. Then, spreading the sample on the plate was carried out using hockey stick on the surface of the agar. The surface of the agar was then allowed to dry before it is moved or inverted into the plates. Each dilution was plated in triplicates, and the plates were incubated at 37˚C for 48h, 72h, and 96h. All colonies on replicate plates were counted and expressed as mean V cholera CFU per (g/ml).

B. Identifying Colony-Forming Unit (CFU) Using Counting Bacteria Growth on Selected Agar

After the incubation process, a count on the bacteria colonies in each plate was made. While, holding the plate into a light source, the colonies were counted by marking their position on the back of the petri plates with a marking pen. This aid was useful in keeping track of those colonies previously counted in order to avoid recounts. If a plate has more than 300 colonies, it was recorded as TNTC (too numerous to count). From the plate count data, the concentration of bacteria in the original sample was calculated. For statistical reasons, only data from plates which have between 30 and 300 colonies in the calculation were

used. Each colony forming unit (cfu) represents a single cell or a group of cells attached together and are inseparable by shaking. Therefore, the number of cfu in the original sample is determined by multiplying the number of colonies on a dilution plate by the corresponding dilution factor. For example, if there were 200 colonies on the 10^{-4} plate, then there are 200 x10,000 = 2,000,000 colonies or 2 x 10^6 cfu/ml in the original sample. Generally, replicates of each dilution are plated, and the mean count is recorded. Thus, the mean of the data from all groups in the lab would be an excellent estimate of the number of bacteria in the original sample.

All plates were incubated in an incubator at 37° as shown in figure 2 below. After incubation, the plates were counted on a standard colony counter as per the manufacturer's instructions and the results were recorded as number of colony forming units (CFU). For colony enumeration, all colonies were counted on day two, three and four. The number of the Colony Forming Units were reported as $\log_{10}$ CFU per cm^2.

Fig. 2 Images of Agar Plate Were Incubate in Incubator Machine at 37°C

C. Growth Requirements in Microorganisms of CEFCT Bait

A characteristic of microorganisms is their ability to grow and form a population of organisms. One of the results of microbial metabolism is an increase in the size of the cell. Part of the several requirements for successful growth include both chemical and physical.

Chemical Requirements for Growing Microorganism: In order to grow microorganisms successfully, they must have abundant supply of water as well as numerous other substances which include mineral elements, growth factors, and gas, such as oxygen. Virtually all chemical substances in microorganisms contain carbon in some form, whether they are proteins, fats, carbohydrates, or lipids. Perhaps 50 percent of a bacterium's dry weight is carbon. Carbon can be obtained from organic materials in the environment, or it may be derived from carbon dioxide. Both chemoautotrophic and photoautotrophic microorganisms obtain their energy and produce their nutrients from simple inorganic compounds such as carbon dioxide. Chemoautotrophs do so through chemical reactions, while photoautotrophs use photosynthesis. Among the other elements that are required by microorganisms are nitrogen and phosphorous. Nitrogen is used for the synthesis of proteins, amino acids, DNA, and RNA. Bacteria that obtain nitrogen directly from the atmosphere are called nitrogen-fixing bacteria. They include species of Rhizobium and Azotobacter, both found in the soil. Phosphorusis is an essential element for nucleic acid synthesis and for the construction of phospholipids. Oxygen is used by aerobic bacteria during the process of cellular respiration as a final electron acceptor. For aerobic organisms, oxygen is an absolute requirement for their energy-yielding properties. Certain microorganisms grow in oxygen-free environments and are described as anaerobic. Organisms such as these, produce odoriferous gases in their metabolism including hydrogen sulfide gas and methane. Certain pathogenic species, such as Clostridium species, are anaerobic, while, certain species of microorganisms are said to be facultative. These species grow in either the presence or absence of oxygen. Some bacteria species are microaerophilic, implying that they grow in low concentrations of oxygen. In some cases, these organisms require an environment that is rich in carbon dioxide. Such kinds of organisms are said to be capnophilic. Other chemical requirements for microbial growth include such trace elements as iron, copper, and zinc. These elements are often used for the synthesis of enzymes. Organic growth factors such as vitamins may also be required by certain bacteria. So also, amino acids, purines, and pyrimidines are required to be present.

Physical Requirements in Growing Microorganism: Certain physical conditions affect the type and amount of microbial growth. For example, enzyme activity depends on the temperature of the environment, and microorganisms are classified in three groups according to their temperature preferences. Psychrophilic organisms (psychrophiles) prefer cold temperatures of about 0°C to 20°C; while, mesophilic organisms (mesophiles) prefer temperatures at 20°C to 40°C; and, thermophilic organisms (thermophiles) prefer temperatures higher than 40°C. A minimum and a maximum growth temperature range exists for each species. The temperature at which best growth occurs is the optimum growth temperature. Another physical requirement is the extent of acidity or alkalinity, referred to as the pH of a solution. For most bacteria, the optimum pH is between 6.5 and 7.5. Since the pH of most human tissue is 7.0 to 7.2, these neutrophilic bacteria usually grow well in the body.

Certain bacteria, such as those in sauerkraut and yogurt, prefer acidic environments of 6.0 or below. These bacteria are said to be acidophilic. Molds and yeasts are among other common acidophilic microorganisms. Microbial growth propagates best when the osmotic pressure is ideal. Normally, the salt concentration of microbial cytoplasm is about 1 percent. When the external environment also has a one percent salt concentration, then the osmotic pressure is optimum. Should the external salt concentration rises, as when food is salted, water will flow out of the microbial cytoplasm by osmosis through the cell membrane into the environment, thereby causing the microorganisms to shrink and die. By comparison, if exterior water is free of salt, it will flow through the cell membrane into the cytoplasm of the cell, causing the organism to swell and burst. Microorganisms that live in marine environments can tolerate high salt concentrations. These organisms are said to be halophilic. They include diatoms and dinoflagellates, which are two types of unicellular algae that lie at the base of oceanic food chains. There are many other species of halophilic bacteria, fungi, protozoa, and algae.

Precautions in Growing Microorganism: In addition, some guidelines and safety measures should be provided for the growth of bacteria, and also to be colonized well so that contamination does not occur. The sterile petri dishes should be kept closed until when it is ready for pouring agar onto them as air-borne contaminants can easily invade an open Petri dish. Although pre-poured agar plates are available, one can make agar plates from tablet, powdered, or bottled agar by following a few simple instructions. Agar kits usually come with detailed instructions on how to prepare plates, and below are sample procedures for reference. When in doubt, be sure to clearly read the instructions and ask for help if needed (either consult a teacher or call the technical help line of the agar kit supplier). When stirring the broth solution, one should give special consideration to starting the stir scale at a low setting and increasing more speed subsequently. When heating the broth, make sure to cover the flask in such a manner that it will not lend itself to boiling over, but to avoid spillage. When pouring the broth, be cautious in filling up the Petri dish so as not to burn oneself. Hence, it is imperative that one ensures that in this process the Petri dish is covered immediately to allow the substance to cool proportionately. Once the Petri dishes have been exposed or inoculated, it should not be re-opened again. For incubation, place each Petri dish inside a zip lock bag to prevent drying out and to control odour. Turn the plates upside down and put them in a warm place. For many microorganisms, the ideal temperature for incubation is 32°C or 90°F. Bacterial growths start to become visible in 2-3 days.

Colony Characteristics on Selected Agar

D. Composition, Uses and Colony Characteristics on Selected Agar

Eosin Methylene Blue (EMB) Agar: Eosin methylene blue agar (EMB) is a selective and differential medium used to isolate fecal coliforms. Eosin Y and methylene blue are pH indicator dyes which combine to form a dark purple precipitate at low pH. They also serve to inhibit the growth of most Gram-positive organisms. Sucrose and lactose serve as fermentable carbohydrate sources which encourage the growth of fecal coliforms and provide a means of differentiating them. Vigorous fermenters of lactose or sucrose will produce quantities of acid sufficient to form the dark purple dye complex.

The growth of these organisms appear dark purple to black. Escherichia coli, a vigorous fermenter, often produces a green metallic sheen. Slow or weak fermenters will produce mucoid pink colonies. Normally-colored or colourless colonies indicate that the organism ferments neither lactose nor sucrose and is not a fecal coliform. Enterobacter aerogenes produces large colonies which are pink to buff around dark centres. Escherichia coli produces small, dark colonies with a green metallic sheen. Pseudomonas, Proteus, Salmonella and Shigella sp produce colourless colonies because they do not ferment lactose.

Fig. 3 Two Examples of Pseudomonas sp. That Grow on the Eosin Methylene Blue (EMB) agar

de Man, Rogosa and Sharpe (MRS) Agar. MRS is de Man, Rogosa and Sharpe; a type of staph bacteria that is resistant to several antibiotics. In the general community, MRS mostly often causes skin infections. In some cases, it causes pneumonia (lung infections) and other issues. If left untreated, MRS infections can become severe and cause sepsis - a life-threatening reaction to severe infection in the body.

Lactobacilli MRS Agar was developed by researchers. deMan, Rogosa and Sharpe as an alternative non-selective media for the cultivation of fastidious lactobacilli. Previous media for the cultivation of lactobacilli employed the use of tomato juice. However, tomato juice agar was undesirable because of its variability and difficulty in preparation. A media described by Rogosa, Mitchell and Wiseman, although adequate for most lactobacilli, however, was found to be unsatisfactory for use with some dairy lactobacilli organisms.

Bearing that in mind, deMan, Rogosa and Sharpe endeavoured and developed a new as well as general non-selective growth medium for lactobacilli. They found that the inclusion of Tween ®80, citrate and acetate resulted in improved growth for lactobacilli. Manganese and magnesium are inorganic ions necessary for growth in the presence of citrate. This media shows a low degree of selectivity, therefore, secondary accompanying microflora may grow well and compete for nutrients. However, most of these accompanying microorganisms can be inhibited by the addition of various concentrations of selective agents, such as cycloheximide, polymyxin, thallium acetate, sorbic acid, acetic acid, or sodium nitrite to the medium.

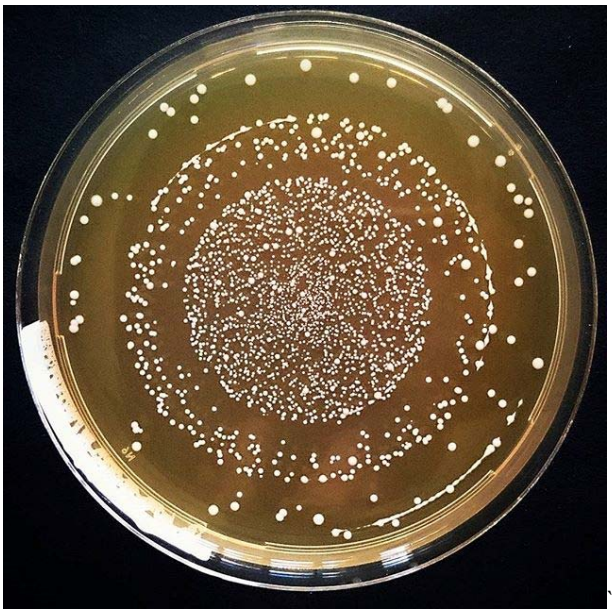
Lactobacilli are long, slender, non-sporeforming, gram-positive rods that are generally facultatively anaerobic, most of which grow well with reduced oxygen tension and increased CO 2. Lactobacilli are important microorganisms for the dairy, food, and

beverage industry. Microbial spoilage of fruit juice is most commonly due to acid uric organisms such as lactic acid bacteria and yeast. Lactic acid organisms are important to the dairy industry for determining the cause of acid defects in dairy products as well as in evaluating the lactic starter cultures in cured cheese and cultured milks.

Lactobacillus brevis is a contaminating organism in the production of beer that, if present, can be responsible for its spoilage. These lactobacilli damage beer by causing turbidity and poor taste due to diacetyl; a strongly flavoured by-product of their metabolism. Finally, lactobacilli are used by the vegetable food industry for the fermentation of cabbage to sauerkraut.

This product may contain components of animal source. Certified knowledge of the origin and/or sanitary conditions of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handled observing the usual universal blood precautions. It is also advised that it should not be ingested, inhaled, or allowed to come into contact with the skin. Similarly, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification. Lactobacilli MRS Agar has a low degree of selectivity allowing for the growth of other lactic acid organisms such as Pediococcus and Leuconostoc species.

Lactobacilli MRS Agar with cycloheximide can be used to inhibit the overgrowth of accompanying microbial flora such as yeasts and moulds. It is important to maintain the appropriate moisture content of the plates during incubation. It is advisable not to allow the surface of the plates to dry out as this will inhibit the growth of lactobacilli due to an increasing concentration of acetate on the agar surface.



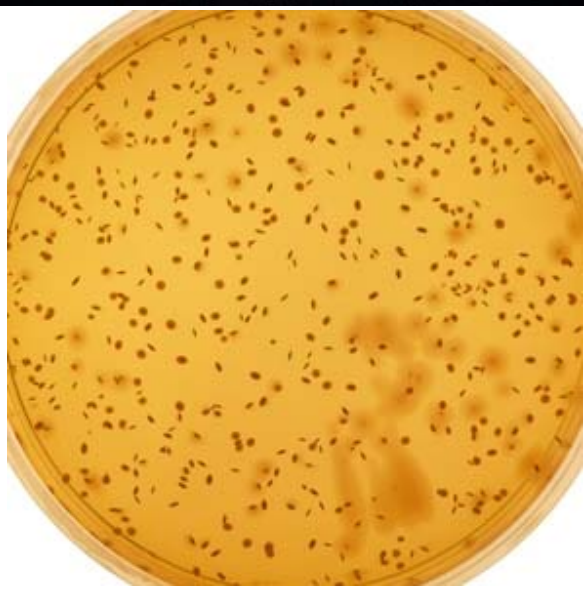


Fig. 4 Two examples of bacteria colonies that grow on the de Man, Rogosa and Sharpe (MRS) agar

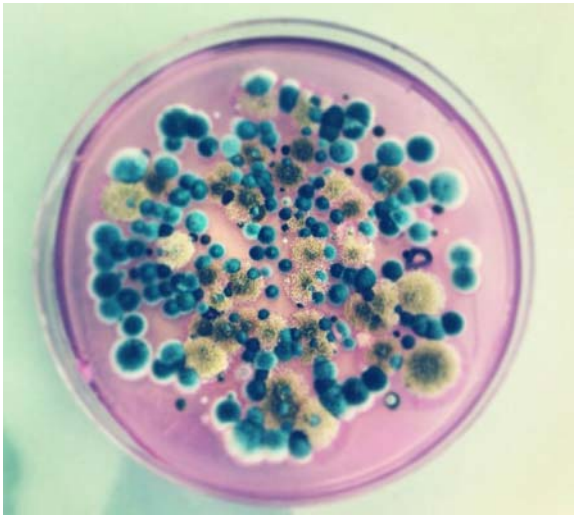
Dichloran Rose Bengal Chloramphenicol (DRBC) Agar. Fungi are recovered from air, soil, lakes, ponds, rivers, streams, waste waters, and good waters. Due to their heterotrophic nature and their ability to adapt to a wide range of environmental conditions, fungi are also frequently encountered as contaminants in various commodities, including foods, inadequately cleaned food processing equipment, and food storage facilities. Since yeasts and moulds can stimulate growth over a wide pH and temperature range, growth can occur on almost any type of food, including processed foods and food ingredients.

Smith and Dawson found that rose bengal added to a near-neutral medium (pH of 6.8) allowed for more colonies to develop than did an acidified medium such as Sabouraud Dextrose Agar. Traditionally, low pH media are used to enumerate yeasts and moulds from water, soil, and food. Such media are now believed to be inferior to selective media with antibiotics. The use of antibiotics for suppressing bacteria, rather than acid, results in improved recovery of injured (acid-sensitive) fungal cells, better control of bacteria, and less interference during counting from precipitated food particles.

Hardy Diagnostics DRBC Agar contains peptone as a source of carbon and nitrogen, dextrose as an energy source, and magnesium sulfate to provide trace elements. The medium contains chloramphenicol, which is added to inhibit most bacterial growth. In addition to chloramphenicol, rose bengal is added to increase the selectivity and to help control overgrowth of rapidly growing moulds such as Neurospora and Rhizopus species. Dichloran is added to the media to inhibit the spreading of moulds by reducing colony diameters. DRBC Agar conforms to the APHA guidelines for the mycological examination of foods.

This product may contain components of animal source. Certified knowledge of the origin and/or sanitary condition of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handled observing the usual universal blood precautions. It is also advisable not to ingest, inhale, or allow to come in close contact with the skin. Ensuring to sterilize all biohazard waste before disposal is also recommended.

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification. It is important to protect this medium from light since photodegradation of rose bengal produces compounds that are toxic to fungi. Due to the selective nature, some strains may grow poorly or fail to grow at all on this medium. Then, Chloramphenicol may not be sufficient to inhibit all bacterial floras.



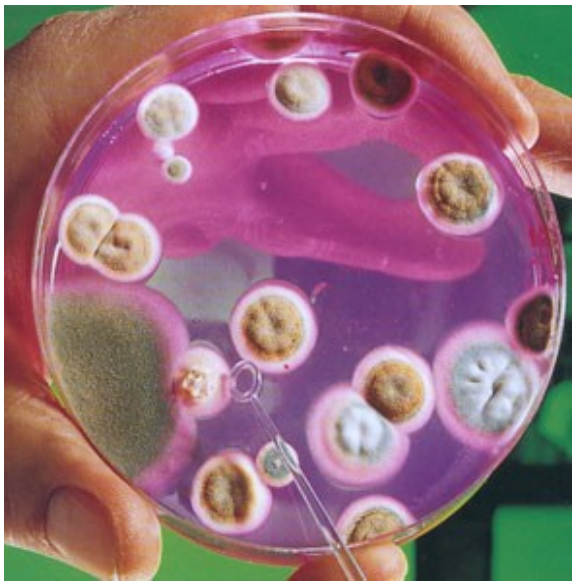


Fig. 5 Two Examples of Agar Containing Moulds That Grow on the Dichloran Rose Bengal Chloramphenicol (DRBC) Agar

III. Result and Discussion

This section presents the result of data analysis using the data obtained from the experiment conducted on selected agar.

A. Data collected from CEFCT Sample Microorganism

TABLE I

Types of Media / Times of Bacteria Growth (Hours)	Day 2, 3, and 4							
	0.3 H	2 H	4 H	6 H	8 H	12 H	Control CEFCT Powder	Control Molasses
Methichillin Resistant Staphylococcus (MRS)	0	0	0	0	0	0	0	0
Eosin Methylene Blue Agar (EMB)	0	0	0	0	0	0	0	0
Dichloran Rose Bengal Chlortetraccyline (DRBC)	0	0	0	0	0	0	0	0

B. Result of CEFCT Sample on Eosin Methylene Blue (EMB) Agar

Therefore, based on observations during the four days that the experiment was carried out, conclusion can be made that there are no features on the growth of bacteria on agar Eosin Methylene Blue (EMB) as shown in figure 6. This indicates that the bait

is safe to be used without posing any threat to wildlife and humans when used to trap flies, since, there is no fecal coliform present in the materials used to produce the bait. The presence of fecal coliform in aquatic environments may indicate that the water has been contaminated with the fecal material of humans or other animals. Fecal coliform bacteria can enter rivers through direct discharge of waste from mammals or birds, or from agricultural or storm runoff, and from human sewage. However, their presence may also be as a result of plant material, or pulp or paper mill effluent. Equally, faulty home septic systems can allow coliforms in the effluent to flow into the water table, aquifers, drainage ditches and nearby surface waters.

Significantly, sewage connections that are connected to the storm drain pipes can also allow human sewage into surface waters. Some older industrial cities, particularly in the Northeast and Midwest of the United States, use a combined sewer system to handle waste. A combined sewer carries both domestic sewage and storm water. During high rainfall periods, a combined sewer can become overloaded and overflow to a nearby stream or river, thereby, bypassing treatment. More so, pets, especially dogs, can contribute to fecal contamination of surface waters. Runoff from roads, parking lots, and yards can carry animal wastes to streams through storm sewers. Birds equally can be a significant source of fecal coliform bacteria. Swans, geese, seagulls, and other waterfowl can all elevate bacterial counts, especially in wetlands, lakes, ponds, and rivers. So also, agricultural practices such as: allowing livestock to graze near water bodies; spreading manure as fertilizer on fields during wet periods, using sewage sludge biosolids; and, allowing livestock watering in streams; can all contribute to fecal coliform contamination.

Therefore, there are some problems that result from fecal contamination of water which are, large quantities of fecal coliform bacteria in the water are not harmful according to some authorities, but may indicate a higher risk of pathogens being present in the water. Some waterborne pathogenic diseases that may coincide with fecal coliform contamination include ear infections, dysentery, typhoid fever, viral and bacterial gastroenteritis, and hepatitis A. Untreated organic matter that contains fecal coliform can be harmful to the environment. Aerobic decomposition of this material can reduce dissolved oxygen levels if discharged into rivers or waterways. This may reduce the oxygen level that is enough to kill fish and other aquatic life. Reduction of fecal coliform in wastewater may require the use of chlorine and other disinfectant chemicals. Such materials may kill the fecal coliform and disease bacteria. They also kill bacteria that are essential to the proper balance of the aquatic environment, thereby endangering the survival of species dependent on these bacteria. Thus, higher levels of fecal coliform require higher levels of chlorine for threatening of those aquatic organisms.

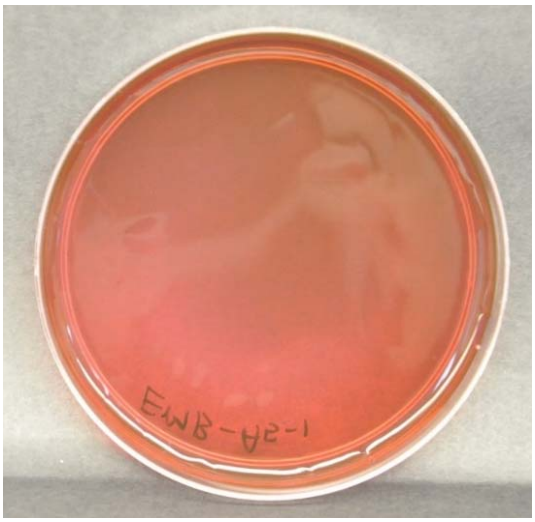


Fig. 6 Image of Eosin Methylene Blue (EMB) Agar After Four Days Incubation Process

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C. Result of CEFCT Sample on de Man, Rogosa and Sharpe (MRS) Agar

However, based on observations during the four days that the experiment was carried out, conclusion could be deduced that there are no features on the growth of bacteria on agar de Man, Rogosa and Sharpe (MRS) as shown in figure 7. This indicates that the bait is safe to be used free of any threats to wildlife and humans when used to trap flies because no Lactobacillus was included in the materials in producing the bait, as it could lead to harmful side effects, if it was not avoided such as, Lactobacillus acidophilus.

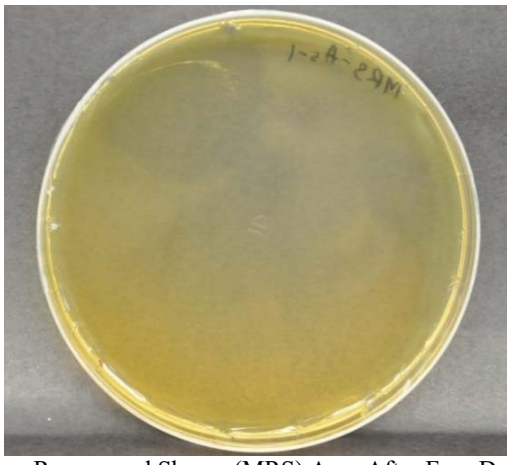

Fig. 7 Image of de Man, Rogosa and Sharpe (MRS) Agar After Four Days Incubation Process

L. acidophilus is likely to be safe when used appropriately in the general population of children and adults. L. acidophilus has been well tolerated, with few side effects reported. The most common side effect is gas, which usually decreases with continued use. Though some Lactobacillus strains have been linked to infections, the probiotic use of these strains is generally considered safe. Some researchers suggest that half of the reported serious cases of infections due to Lactobacillus tend to occur in people with immune problems.

Lactobacillus may also cause arthritis, bloating, blockage of a lung artery, diarrhea, disease of the esophagus, heart inflammation, liver infection, skin reactions, stomach cramping, stomach lining inflammation, stomach rumbling, and vaginal burning and discomfort.

It should be used cautiously in people with stomach disorders. High doses (over 109 cells daily) have been linked to mild stomach problems. Equally, it should be used cautiously in people with short bowel syndrome. Lactobacillus may cause (bacteremia), a condition where bacteria are found in the blood. It should be used cautiously in people who have high fever. It should be used cautiously in people who have fixed orthodontic appliances (i.e., braces), chewing problems, or misaligned teeth, as L. acidophilus may cause tooth decay. Similarly, it should be used cautiously in infants and children. L. acidophilus supplementation in the first six months of life may increase the risks of allergies to cow milk in some children. Although L. acidophilus has been shown to reduce colic in infants, there is no enough evidence about the safety of long-term L. acidophilus used in this age group. Bacteremia and sepsis, or shock, have been reported after pediatric use of probiotics.

There is a report of severe dehydration in a child with diarrhea after treatment with rehydrating solution and Lacteol by mouth. However, it should be avoided if one is allergic or sensitive to Lactobacillus acidophilus, its parts, or members of the Lactobacillaceae family. It should also be avoided in people who suffer milk allergies, due to possible milk allergens in L. acidophilus preparations made from dairy products. Similarly, it should be avoided in people who have immune problems, as Lactobacillus may cause diseases.

Lactobacillus has been used safely during pregnancy (2-4 weeks before childbirth) and breastfeeding (for up to six months). L. acidophilus vaginal tablets have been studied in pregnant women for the treatment of bacterial vaginosis. An L. acidophilus-containing culture (Narine) has been studied in pregnant women for treatment of suppuratives inflammatory disease, with no side effects. Studies have found that higher Lactobacilli levels in the vagina may be linked to a lower risk of premature childbirth, and L. acidophilus has been studied in pregnant women to prevent premature childbirth.

D. Result of CEFCT Sample on Dichloran Rose Bengal Chloramphenicol (DRBC) Agar

Therefore, based on the observations during the four days of the experiment that it was carried out, conclusion can be drawn that there are no features on the growth of bacteria on agar Dichloran Rose Bengal Chloramphenicol (DRBC). This indicates that the bait is safe to use without threat to wildlife and humans when used to trap flies because no yeasts and moulds included in the materials in producing the bait as it can lead to food spoilage through contamination of yeasts and moulds.

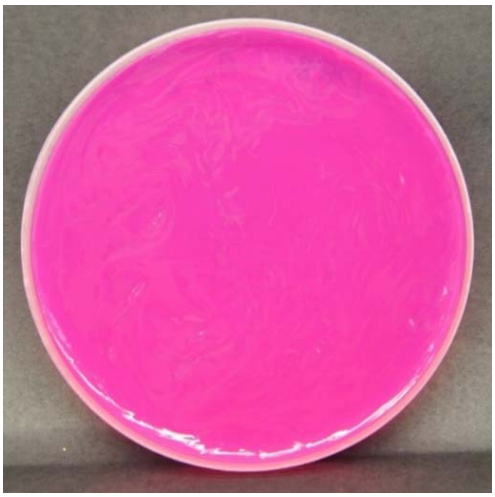

Fig. 8 Image of de Man, Rogosa and Sharpe (MRS) Agar After Four Days Incubation Process

Moulds classified as fungi develop a multicellular structure visible to the naked eye. They grow from cells called spores present in the air. They settle and multiply on suitable foods. At this stage, they are visible as a fluffy coloured mass and the food is said to have gone mouldy. Moulds grow most readily in most conditions, at temperatures between 20oC and 40oC. They grow on a variety of foods, particularly meat, cheese, fruit and bread, especially if the food is stored in damp conditions. Moulds may remain active at low temperatures of a refrigerator but they are destroyed by heat above 70oC. They are also like a slightly acid medium, hence, the reason they attack citrus and the surface of jams.

Food that is contaminated with mould often appears to be safe to be eaten as only the outer part is affected by mould growth. However, recent research has shown that substances produced by the mould which migrate into the food could be harmful to many organs of the body. It is therefore advisable to discard mouldy food completely, rather than just to remove the mouldy part.

Yeasts are microscopic fungi. Yeast cells grow and reproduce in conditions similar to those required by other fungi. They need oxygen, warmth, food and moisture in order to grow successfully. Yeasts grow well at temperatures between 25oC and 30oC. Extreme heat destroys all yeasts and most are destroyed at temperatures above 60oC. They are found in the air and soil, and on the surface of fruit. Some are able to tolerate fairly high acidic, salt and sugar concentrations and can grow without the presence of oxygen. The activity of yeast is used in the baking and brewing industries to make bread, doughnut and alcoholic beverages through a process called fermentation. However, they can cause food spoilage in syrups, fruits, fruits juices and jam especially as they can survive without air.

Yeast cells reproduce by budding. At first, a small projection appears at the edge of the parent cell, and from this, cytoplasm and nutrients develop. As the bud grows, the nucleus moves towards it and splits, such that a new nucleus enters the bud. When the bud is almost as large as the parent cell, a wall forms that separates it from the parent cell, and it then breaks away. When they are reproducing rapidly, the buds do not break away but continue to reproduce until long chains of yeast cells are formed.

IV. Conclusions

Researchers conducted a hygienic study on CEFCT bait to determine the safety of the bait for use in Malaysia. Hence, laboratory test was conducted on three types of media like Eosin Methylene Blue (EMB) Agar, de Man, Rogosa and Sharpe (MRS) Agar, and Dichloran Rose Bengal Chloramphenicol (DRBC) Agar to examine the microorganism growth. Based on the results from all experiments, the study found that CEFCT bait does not grow any microorganism in the selected media. This indicates that the bait is safe to use without posing a threat to wildlife and humans when used to trap flies as no fecal coliform was included in the materials in producing the bait, since the presence of fecal coliform in aquatic environments may indicate that the water has been contaminated with the fecal material of humans or other animals. Therefore, it can be concluded that CEFCT baits are safe to be used not only in Malaysia but all over the world.

Further studies are necessary in order to find low-cost bait components with similar effects of the best bait (CEFCT) in local markets in order to develop new low-cost baits. To identify the active compounds of the CEFCT baits, volatile analysis of CEFCT bait can be done.

Nomenclature

cfu	colony forming unit
log	logarithm

CO_2 Carbon Dioxide
DNA Deoxyribonucleic Acid
DH_2O Distilled Water

Greek letters
µl Microliter

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