Isolated Effective Microorganism(EM : *B.subtilis*) from Natural to be Green Material for Environmental Management in Wastewater Treatment

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Abstract

Effective Microorganism (EM) has developed in Okinawa, Japan in 1970 and useful for green environmental management. Study on microorganism isolation from 4 areas of wastewater collection as found *B.subtills* by series steps beginning with cell culture. After Gram staining, the 16S rRNA sequences of a total of *B.subtills* were verified by PCR. The *B.subtills* was tested in laboratory, the results of testing showed that the good conditions for *B.subtills* growing as pH 3-7, salinity 30-70 ppt and temperature between 50-80 ^{o}C. Wastewater from Tapioca Starch Industry was tested by *B.subtills* added and compared with control that no added *B.subtills* in wastewater. The results showed a significant decreased in BOD in wastewater with *B.subtills* added and wastewater without *B.subtills* added as 47.73% and 19.59% respectively.

Key words : EM, *B.subtills* ,BOD, wastewater

1. Introduction

Effective Microorganism(EM) , a culture of coexisting beneficial microorganism predominantly consisting of lactic acid bacteria, photosynthetic bacteria, yeast, fermenting fungi and actinomycetes Namasivayam and Kirithiga(2010). Chaudhry and Lqupal (2006) explained that Effective Microorganisms solutions contain a mixture of five genera of organisms, namely Actinomycetes, Ray fungi, Photosynthetic bacteria, Yeasts and Lactic acid bacteria. The concept of Effective Microorganisms was developed by Japanese researcher named is Teuro higa from the University of Ryukyus, 80 different microorganisms have positivity influencing decomposing organic matter. EM may have a number of applications, including agriculture, livestock, gardening lanscapeing, composting, bioremediation, cleaning septic tanks, algal control and house hold uses(Higa,2003). Nathan and Robert(2003) studied about EM in wastewater systems and the results showed that experiment was undertaken to determine if the application of EM would decrease the valumes of sewage sludge produced in on-site wastewater treatment systems(septic tanks). Diver(2002) was suggested that the main apecies of EM including : one is Lactic acid bacteria(*Lactobacillus plantarum, L. Casei, Streptoccus lactics*), two is Photosynthetic bacteria(*Rhodoseudomonas palustrus*, *Rhodobacter spaeroides*), three is Yeasts (*Saccharomyces cerevisae, Candida utilis*), four is Actinomycetes (*Streptomyces albus, S.griesus*) and five is Fermenting fungi

(*Aspergillus oryzae, Mucor heimalis*).

The objectives of this research were (i) to isolate EM (*B. subtilis)* from under-treated wastewater by culturing techniques (ii) to identify EM(*B. subtilis)* by classical microbiological tests and PCR(16s rRNA) and testing the effectiveness of EM (*B. subtilis)* in wastewater under the BOD reducing condition.

2. Material and Methods

2.1 Basic information about Bacillus subtilis (*B. subtilis)*

Bacillus subtilis cells are rod-shaped, Gram-positive bacteria that are naturally found in soil and vegetation. *Bacillus subtilis* grow in the mesophilic temperature range. The optimal temperature is 25-35 degrees Celsius (Entrez Genome Project). Stress and starvation are common in this environment; therefore, *Bacillus subtilis* has evolved a set of strategies that allow survival under these harsh conditions. One strategy, for example, is the formation of stress-resistant endospores (wiki, 2010).

B. subtilis is a model organism used to study bacterial chromosome replication. Replication of the single circular chromosome initiates at a single locus, the origin (*oriC*). Replication proceeds bidirectionally and two replication forks progress in clockwise and counterclockwise directions along the chromosome. Chromosome replication is completed when the forks reach the terminus region, which is positioned opposite to the origin on the chromosome map. The terminus region contains several short DNA sequences (*Ter* sites) that promote replication arrest. Specific proteins mediate all the steps in DNA replication. Comparison between the proteins involved in chromosomal DNA replication in *B. subtilis* and in *Escherichia coli* reveals similarities and differences. Although the basic components promoting initiation, elongation, and termination of replication are well-conserved, some important differences can be found (such as one bacterium missing proteins essential in the other). These differences and strategies that various bacterial species have adopted to carry out the duplication of their genomes (Narrot, 2006).

Only one DNA molecule is present in these cells. *Bacillus subtilis* has one circular chromosome. The total size of all the DNA is 4,214,814 bp (4.2 Mbp) (TIGR CMR). 4,100 genes code for proteins. 53% of the protein-coding genes are only seen once, while 25% of the genome relates to families of genes that have undergone gene duplication(Kunst1997). Another strategy is the uptake of external DNA, which allow the bacteria to adapt by recombination. However, these strategies are time-consuming. *Bacillus subtilis* can also gain protection more quickly against many stress situations such as acidic, alkaline, osmotic, or oxidative conditions, and heat or ethanol(Microwiki, 2010). The alternative sigma factor ?B is a global regulator of stress response. Heat, acid, or ethanol and glucose or phosphate starvation are all stimuli that activate B (Bandow 2002).

2.2 Methodology

Wastewater samples were taken from 4 areas in Bangkok as Samsean Canal, Lumlooka Canal, Victory monument canal and starch industry wastewater from October 2010 to December 2010. The process of experiments is shown in Fig1.

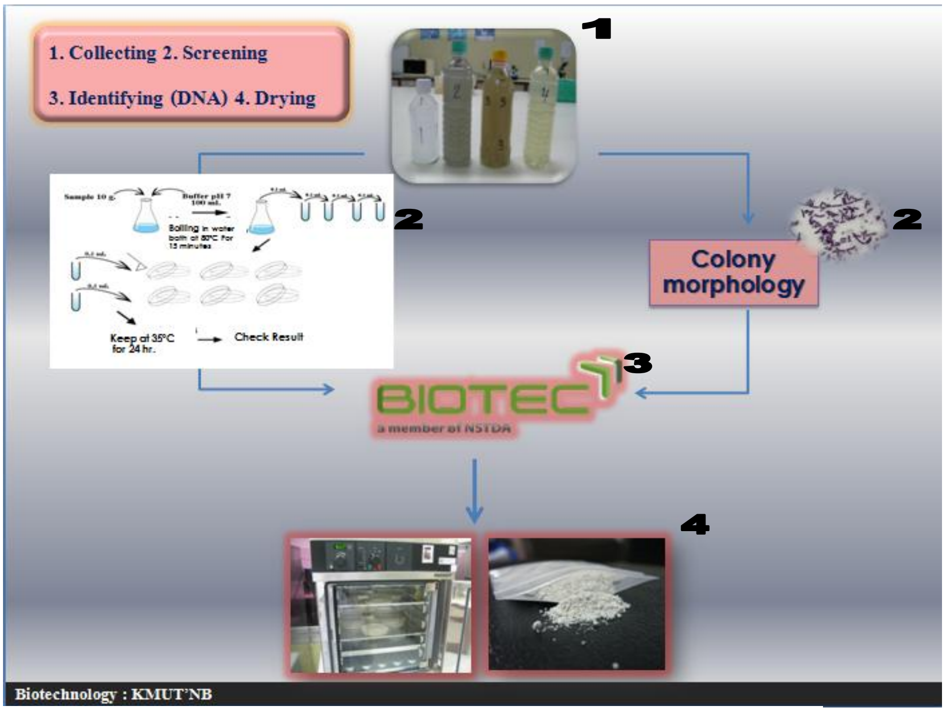

Figure1 The process of screening and isolation EM (*B. subtilis*)

The samples were prepared for DNA isolation, DNA sequencing and DNA analysis. All samples were tested at BIOTECH standard laboratory (www.biotech.or.th). The physical/chemical conditions of EM (*B. subtilis*) were tested as pH, salinity and temperature at Biotechnology laboratory in King Mongkut University of Technology North Bangkok. The efficiency of EM (*B. subtilis)* was tested by BOD reducing from wastewater as following: treatment one was wastewater + EM (*B. subtilis*) added and treatment two was wastewater without EM (*B. subtilis*) added. Experimental data were analyzed by analysis of variance (ANOVA) and Duncan's New Multiple Range (DMRT) at significant 5%.

3. Results and discussion

3.1 *B. subtilis* isolation

The isolated microorganisms were characterized and identified that *B. subtilis* as gram positive mobile rods. The Victory monument canal was the area found *B. subtilis* in wastewater more that the other areas in this experiment because at the Victory monument canal was the spill way of sewage from city. The illustrate of *B. subtilis* as shown in Fig. 2.

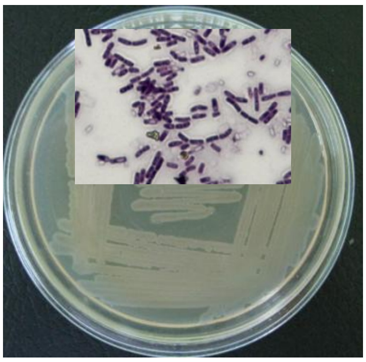

Figure 2 The illustrate of *B. subtilis* isolated in laboratory

3.2 pH condition of *B. subtilis*

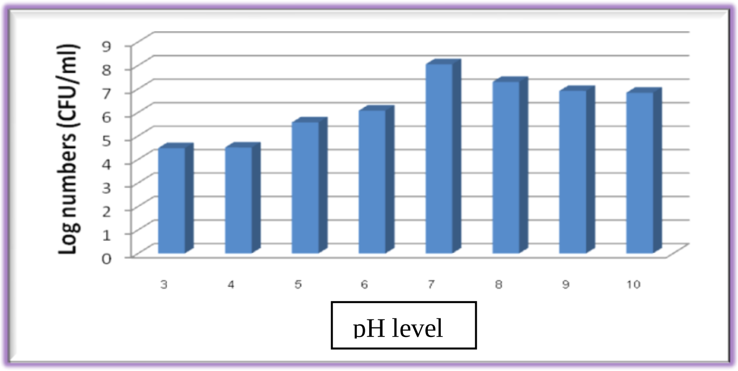

Figure 3 pH level effect to EM*(B. subtilis)*

Fig3. shows the condition of EM (*B. subtilis*) were growing in the different pH level. The lower or higher pH was not suitable for EM (*B. subtilis*) growing. The good environment for EM (*B. subtilis*) growing was pH=7. In addition, Linich (2001) also suggested that a change in microbial populations may impact upon the pH.

3.3 Salinity condition of EM *(B. subtilis)*

Analysis of salinity by monitoring population of EM *(B. subtilis)* living in different levels of salinity as 30,40,50,60 and 70 ppt. respectively. The results showed that populations of EM *(B. subtilis)* were living more in the lower level of salinity. At 30 ppt. of salinity found the highest population and at 70 ppt. of salinity found the lowest EM *(B. subtilis)* growing in this condition. The salinity level condition and effect to EM *(B. subtilis)* as shown in Fig 4.

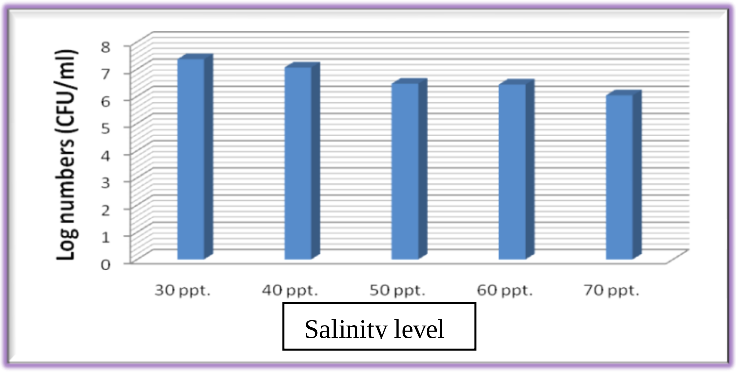

Figure 4 Salinity level effect to EM *(B. subtilis)*

3.4 Temperature condition of EM *(B. subtilis)*

The growth of EM *(B. subtilis)* was observed at temperature 50-80^{o}C. The EM *(B. subtilis)* may grow in the lower temperature less than 50 ^{o}C but from the experiments found a bit of microorganism growing. The good condition of EM *(B. subtilis)* growing at temperature 50 ^{o}C based on the results obtained from this experiment, it was conducted that the use of EM *(B. subtilis)* had minimum effected on the environments were taken from. The temperature level effect to EM*(B. subtilis)* as shown in Fig5.

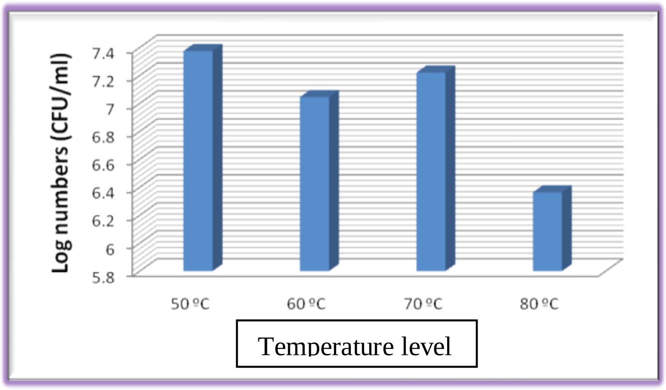

Figure 5 Temperature level effect to EM *(B. subtilis)*

3.5 The efficiency of EM (*B. subtilis*) in wastewater

BOD is one of index parameter for water quality testing, the low value of BOD shows that water is good quality. BOD is stand for Biochemical Oxygen Demand and the maximum effluent concentration for BOD_5 at 20°C is specified by the government at 20 mg/L. and all industries are held to less stringent standards. The comparison of BOD by testing EM (*B. subtilis*) in two samples of wastewater collected from starch industry. Sample one was wastewater with EM added and sample two was wastewater without EM added. The results of testing as shown in Fig. 6.

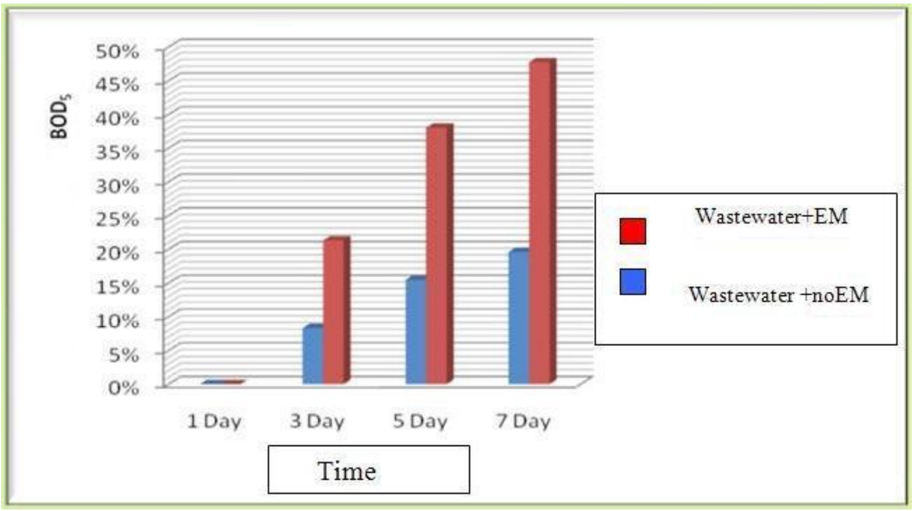

Figure 6 The efficiency of EM (*B. subtilis*) in wastewater

The BOD_5 of the EM (*B. subtilis*) treated groups was found to be significantly reduced by 47.73% (Wastewater with EM (*B.subtilis*) added) and 19.59% (Wastewater without EM (*B. subtilis*) added) at 95% confidence level respectively when compared with the untreated control. The EM (*B. subtilis*) treated groups showed an increase in pH, reduction in turbidity and odor.

4. Conclusion

From experiments, the 16S rRNA sequences of a total of *B.subtills* were verified by PCR and microorganism as *B.subtills* was selected . The results of testing showed that the good conditions for *B.subtills* growing as pH 7, salinity 30 ppt and temperature between 50 ^{o}C. The effectiveness of *B.subtills* were tested and the results showed a significant decreased in BOD in wastewater with *B.subtills* added and wastewater without *B.subtills* added as 47.73% and 19.59% respectively. Further studies, should be conducted with purpose of achieving BOD level reduction of the other wastewater treatment plants such as from domestic wastewater treatment plant. The other microorganisms in EM groups should be tested as following : Lactic acid bacteria(*Lactobacillus plantarum, L. Casei, Streptoccus lactics*), Photosynthetic bacteria(*Rhodoseudomonas palustrus, Rhodobacter spaeroides*), Yeasts (*Saccharomyces cerevisae, Candida utilis*), Actinomycetes (*Streptomyces albus, S.griesus*) and Fermenting fungi(*Aspergillus oryzae, Mucor heimalis*).

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