

Use of Effective Microorganisms[®] (EM[®]) for Sustainable Pathogen Control in Food Safety.

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Abstract—TeraGanix, Inc. produces and distributes the original and certified brand, Effective Microorganisms (EM) for the United States. EM all-natural liquid probiotic comprises of three groups of microbes: Lactic acid bacteria, yeast, and photosynthetic bacteria. Given that lactic acid bacteria has been used for centuries for food preservation and fermentation, EM has the potential to be used as a biocontrol for common food-borne pathogens such as *Salmonella*, *Listeria*, and *E. Coli*. This paper is a gathering of research done to support the use of EM in pathogen control as it relates to food safety.

Index Terms—Effective Microorganisms[®], EM-1[®], lactic acid bacteria, *Lactobacillus*, *Salmonella*, *Escherichia coli*

I. INTRODUCTION

CONSUMERS are increasing their desire for more natural foods treated with fewer chemicals as well as meat and dairy with fewer antibiotics. Although food safety has improved, opportunities remain to improve food naturally. This is leading to more research in the use of beneficial microorganisms, such as Effective Microorganisms or EM, for pathogen control in areas such as food raising, food preservation, and food safety (2).

TeraGanix, Inc. provides EM Technology and education to help agriculture producers improve its operations naturally through EM. EM can be used to improve the health of animals, resulting in cleaner poultry, beef, and swine operations where *Salmonella* and *E. coli* are generally present. Effective Microorganisms[®] or EM[®] is a brand name referring to a family of beneficial microbial-based products using technology developed by Japanese scientist Dr. Teruo Higa and the intellectual property is owned by EM Research Organization (EMRO) in Okinawa, Japan (9). This paper will focus on research demonstrating pathogen controls in agriculture and environmental treatments with the use of EM Technology as well as *Lactobacillus spp.*

II. BACKGROUND

A. Pathogens in The Food We Eat

According to the Centers for Disease Control and Prevention (CDC), there are 31 major pathogens that cause 9.4 million

incidents of food-borne illnesses in the United States yearly. *Salmonella spp.* is the leading cause of both hospitalizations (35%) and deaths (28%) among the 9.4 million cases (13). When an outbreak does occur, the cost can be substantial for the producer. Between 1994 and 2013, 1,300 meat and poultry recalls were reported (an estimated 638 million pounds of product) For the producer an average of \$109 million in market equity is lost in the 5 days following a recall (14). *Salmonella* in raw produce, poultry (including eggs), pork and tuna have the highest incidences followed by *Listeria monocytogenes* from dairy (23).

Antibiotics have largely been used to control pathogenic bacteria in livestock. There are a few major disadvantages to the overuse of antibiotics; (1) long term use can cause some bacteria to become resistant, and (2) antibiotics may impact the entire bacterial population including beneficial bacteria (21). Alternatives for antibiotics in food safety, such as probiotics are increasingly being considered and researched for their ability to reduce or eliminate pathogens. Probiotics are largely used in livestock operations for their significant positive effects on animal health and productivity (22).

B. What is EM

Effective Microorganisms (commercially known as EM-1[®]) is an acidic (pH 3.5) liquid microbial product consisting of three main groups of beneficial, facultative microorganisms; Lactic acid bacteria (*Lactobacillus casei*), yeast (*Saccharomyces cerevisiae*) and photosynthetic bacteria (*Rhodospseudomonas palustris*). The microbes in EM are alive, but in a state of semi-dormancy. EM is used worldwide in the agriculture industry for dairy, beef, poultry, swine, crop production and waste water treatment (9).

C. EM Pathogen Control in Agriculture

Techniques used to suppress pathogens after slaughter are relatively effective but finding ways to reduce pathogens while raising livestock pre-slaughter can be equally important. Research in the livestock industry has shown that probiotics, such as EM, can reduce or eliminate pathogen populations in animal guts, bedding materials and manure.

One of the most common pathogens associated with poultry is *S. enteric* serovar Typhimurium, which causes an infection in the upper gastrointestinal tract (13). In a study done by C.L. Sheffield et al. (7), sterile poultry manure was inoculated with *S. enteric* serovar Typhimurium and then treated with Lt1000 poultry litter treatment (Lt1000 is TeraGanix's liquid microbial product containing EM[®] for poultry production). In 91% of the

trials, *Salmonella* was completely eliminated within 9 days (see Table 1). A lab study done by Ravishankar Lab at the University of Arizona (30), showed a significant reduction in *Salmonella* Heidelberg and *E. coli* O78:H11 when the pathogens were treated with a 3, 5, 10 and 50 percent EM-1 solution and incubated for 24 and 48 hours (see Tables 2 and 3).

Another study done on poultry by Vicente et al. (15) looked at the colonization of *Salmonella* in birds treated with a *Lactobacillus spp.* (LAB) probiotic. Chicks were inoculated with *Salmonella* and then administered the probiotic for 3 consecutive days. Results showed that after 24hr only 5% of chicks treated tested positive for *Salmonella* while 70% tested positive in the control group. However, continued applications are important since after 72hrs 25% of treated chicks tested positive for *Salmonella* (compared to 65% in the control). Additional studies have shown when poultry is raised on a probiotic, colonization of *Salmonella* is reduced by 70% (16).

M.H. El-Deep et al. (20) studied performance and pathogenic intestinal bacteria of domestic fowl. Chicks were fed EM probiotics starting at 4 weeks until the age of 41 weeks. *E. coli*, *Salmonella*, *Staphylococci* and *Coccidia ovum* were tested in the intestinal tracts. Total viable bacteria of the pathogens were significantly reduced compared to chicks not given EM probiotics. As a result of a more balanced and healthy gut, body weight also increased by almost 18%.

W. Esatu et al (29) tested antibody measurements on Horro and Fayoumi chickens because the exotic breed, Fayoumi, were more prone to infectious diseases compared to local, Horro, chickens. Antibody response is the immune systems ability to fighting infectious disease. Chicks were either given feed, water or feed and water treated with EM from 3 to 8 weeks of age. Antibody responses significantly increased in treated birds regardless of EM treatment (feed/water/both). However, this study also proves the need for continued and repeated treatments throughout the life of the animal. Antibody response peaked at 3 weeks, immediately after treatment, then declined through the remaining weeks. Although antibody count still remained significantly higher in EM treated chicks than the control at 8 weeks.

In dairy cows, mastitis caused by *Staphylococcus aureus* and *Streptococcus aureus* in the utter milk, is a common ailment. The bacteria infect the mammary gland and can quickly become a lingering and painful problem if not treated. A lab trial done by Minako Matsumori (12) on EMs effects on *Staphylococcus aureus* showed complete elimination of the bacteria when EM was applied to the plated colony. After one day, sample No. 1 (EM treated) showed no presence of *Staphylococcus aureus*, whereas sample No. 2 (no EM) had a population of 8.2×10^7 CFU. A second study on dairy using strictly LAB, tested the growth of *E. coli*, *Salmonella*, *Staphylococcus*, *Listeria*, *Enterococcus faecium* and *Enterococcus faecalis* in *Lactobacillus acidophilus*- and *L. casei*-fermented milk. *Listeria* and *Staphylococcus* showed the highest sensitivity in the soluble portion of the fermented milk with 85% and 84% control (17).

D. EM Pathogen Control in Waste Water

Food borne illness does not only stem from the consumption of contaminated foods. Animal manure is a by-product frequently used as an economical and beneficial fertilizer in agriculture crops. Manure, however, can harbor common pathogens such as *Salmonella*, *Staphylococcus*, and *Campylobacter* (8).

An area of pathogen control EM has been very successful in is the treatment of livestock manure as well as human wastewater. Waste is generally measured using its Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Suspended Solids (TSS). High organic content and high BOD causes water to be polluted. Therefore, wastewater has to be treated to reduce BOD, COD and TSS. EM can be used as an alternative to harmful chemicals generally used to treat wastewater. In a study by S. Chantsavang (18) in Thailand, EM was shown to significantly decrease the number of organic solids in pig waste. S. Chantsavang found waste treated with EM reduced BOD by 99.5%, COD by 99.6% and TSS by 99.9% after a month of being treated.

S. Monica et al. (19) found that when sewage was treated for 72 hours, BOD reduced from 374.5 to 55.9 mg/l (85%), COD decreased from 570.4 to 99.8 mg/l (82%) and TSS decreased from 486.6 to 43.3 mg/l (91%). Municipal wastewater studies from around the world have demonstrated the ability of EM to reduce several pathogens. In a study done in Naha City, Japan, *E. coli* was reduced from 12,000 CFU/ml pre-treated to 1300 CFU/ml post-treatment (28). Additional data from Australia show *E. coli* drops from as high as 1M CFU/ml to 1000 CFU/ml (29).

E. The use of *Lactobacillus spp.* In Pathogen Control

The following research supports EMs potential in food safety given the main ingredient in EM-1, *Lactobacillus spp.* (LAB). LAB has been used for centuries in food processing and fermentation as well as heavily researched for pathogen control.

In EM products, Lactic Acid Bacteria produce lactic acid from sugars and other carbohydrates that are produced during fermentation. Food and drinks such as yogurt and pickles have been made with LAB for thousands of years. Lactic Acid Bacteria is a strong sterilizing compound that suppresses harmful microorganisms and promotes the fermentation and decomposition of material such as lignin and cellulose, thereby removing undesirable effects of non-decomposed organic matter (9).

Since LAB is already associated with human food consumption, it is widely known to be safe by the general population. LAB produces antimicrobial products when metabolized such as; organic acids, fatty acids, hydrogen peroxide and diacetyl (a compound naturally found in alcoholic beverages) (2). With LAB being a probiotic, some strains have the potential to help suppress gastrointestinal pathogenic bacteria such as *Helicobacter pylori*, *E. Coli*, and *Salmonella* (3). *Lactobacillus* competitively excludes pathogens by decreasing their viability by lowering the pH and producing lactic acid, hydrogen peroxide and other antibacterial

compounds such as bacteriocins (25).

With food safety however, the main focus is on the production of bacteriocins. Bacteriocins inhibit the growth of certain pathogens commonly associated with food borne illnesses, such as, *Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus spp.* and *Enterococcus spp.* (2). Bacteriocins are produced by various gram-negative and gram-positive bacteria but the bacteriocins in LAB are of particular importance because of their potential in food safety and preservative abilities. Vuyst and Leroy (3) define them as small, ribosomally synthesized, antimicrobial peptides or proteins that possess activity towards closely related gram-positive bacteria (i.e. pathogens).

Salmonella is one of the most common pathogens associated with food safety, in fact 95% of salmonellosis cases are food borne in origin and very frequently from poultry products (7). In a study by L. Makras et al. (4) various strains of *Lactobacillus*, including *L. casei* and *L. plantarum* (two strains found in EM-1), were found to have strong antibacterial activity and significantly inhibit the invasion of *Salmonella* into cultured human Caco-2 cells (intestinal cells found in the colon (5)).

S. Hudault et al. (24) predicted *L. casei* would display an antagonistic effect on bacteria *Salmonella* and *Escherichia coli*. Lab mice were inoculated with *Salmonella typhimurium* and then given a probiotic (*L. casei*). Results showed that after 1 hour of contact between *S. typhimurium* and *L. casei*, invasion was impeded in the Caco-2 cell and populations of *L. casei* established themselves in different areas of the gut.

A study done on broiler chicks evaluated a *Lactobacillus*-based probiotic on chicks inoculated with 10^4 CFU of *Salmonella*. Cecal tonsils samples were taken and compared. When *Lactobacillus* was given orally, only 15% remained in the treated samples, compared to 85% in control (26).

III. INCORPORATING EM INTO AN OPERATION FOR OPTIMAL PATHOGEN CONTROL

All agricultural businesses must deal with issues of food safety in some form or another. Lack of understanding has led many crop farmers to cease the use of any type of animal-based manure, including composts, for fear of a recall due to pathogens.

Livestock operations have lots of manure and wastewater that harbors pathogenic bacteria that increases the risk of animals getting sick as well as the people consuming them. Confined operations are susceptible to fast-moving sicknesses and tend to overuse antibiotics, which increases the growth and resistance of many pathogenic microbes. In some areas irrigation water can be contaminated with pathogens and contaminate the soils where crops are grown. Packing houses receive foods that can have bird or rodent droppings that can breed when put into packaging. Food processing facilities can also have issues in and around their facilities from rotting foods and wastewater.

TeraGanix, Inc recommends the regular use of Effective Microorganisms probiotic products throughout the livestock operation for the best results in pathogen control. TeraGanix

has developed a line of ready to use (Activated EM•1) products that are labeled for specific applications. The series has a letter and number sequence such as “Ag” for “Agriculture” followed by a number such as “1000”. The concept that was developed decades ago by the developers of EM was a very pragmatic and regular approach of applying the products at each part of any operation. These regular applications build up beneficial microbial populations and begin to create a probiotic environment that suppresses odors and pathogens, essentially cleaning or bioremediating the operation.

Starting in 2010, TeraGanix began using this naming system and testing these ready to use products. In other countries these products are referred to as “activated EM•1”. The first of these was the 2014 study by Dr. C. Sheffield et al. (7) on Lt1000®, demonstrating *Salmonella* suppression on a commercial broiler operation in East Texas. Litter from houses with 22,000 birds were tested for the presence of *Salmonella*.

Table 1. Alteration in *Salmonella Typhimurium* concentration over time

Trial (cfu/mL)	Positive control	Days postinoculation		
		5	7	9
1	8.70 ¹	5.18	0.00	0.00
2	8.70	8.16	6.64	5.00
3	8.60	7.10	6.50	0.00
4	8.62	7.43	6.50	0.00
Mean ± SD	8.66 ± 0.05 ^a	6.97 ± 1.27 ^b	4.91 ± 3.27 ^b	1.25 ± 2.50 ^b

^{a,b}Values with different superscripts differ significantly as analyzed by ANOVA and Bonferoni's test ($P < 0.05$).

¹Log₁₀ transformed mean (cfu/mL) of 3 replicates, except trial 1, which had only 2 replicates.

This study prompted exploring other food safety-specific microbes including *Listeria* and *E. Coli*. In 2015 a study was done in the food safety lab at the University of Arizona on *Salmonella* Heidelberg and *Escherichia coli* O78:H11 (30). Table 2 shows the reduction in *E. coli* O78:H11 populations and Table 3 show survival of *S. Heidelberg* after EM•1 treatments.

Table 2. Reduction in *E.coli* 078:H11(Log cfu/mL) population after EM treatment

Treatment	24 hours	48 hours
3% EM-1	0.09	0.40
5% EM-1	0.05	0.50
10% EM-1	1.62	1.77
50% EM-1	3.29	5.84

Table 3. Survival of *S. Heidelberg* (Log cfu/mL) in EM solutions

Treatment	0 hour	24 hours	48 hours
TSB control	6.11	9.01	9.16
3% EM-1	-	8.87	8.85
5% EM-1	-	8.75	8.20
10% EM-1	-	7.26	6.12
50% EM-1	-	5.64	3.00

Apc500® is a liquid probiotic cleaner that has a slightly different formulation than the pure microbial products. It is used for cleaning all surfaces including metals, concrete, equipment, and facilities. It is non-corrosive and made of all food grade ingredients. Apc500® deodorizes without harsh chemicals or perfumes and acts as a prebiotic for the other

EM-based products from TeraGanix. Tests at Nova Labs in Conroe, Texas demonstrated 89.3% suppression of MRSA with a single application (27).

Table 5. APC500® 1% vs *Staphylococcus aureus* 99% (MRSA) ATCC 33591

Contact Time	0 Time	30 seconds	5 minutes
Actual Count Replicate 1	2.50x10 ⁵	8.10x10 ⁵	1.62x10 ⁶
Actual Count Replicate 2	6.25x10 ⁵	1.44x10 ⁶	1.52x10 ⁶
Actual Count Replicate 3	8.25x10 ⁵	5.8x10 ⁶	1.43x10 ⁶
Average Count	5.66x10 ⁵	2.68x10 ⁶	1.52x10 ⁶
% Reduction from Numbers Control 5.3x10 ⁶ cfu/mL	89.3%	49.3%	71.2%

These are just a few select samples of in-situ applications in various agricultural situations that demonstrate the potential of regular use of the probiotic formulas to suppress pathogenic bacteria.

IV. CONCLUSION

The purpose of this review is to illustrate the strong potential Effective Microorganisms® has in the control of common food-borne pathogens such as *Salmonella*, *Listeria*, and *E. Coli*. EM has the possibility of providing agricultural producers with a safe, all-natural and chemical free option to pathogen control that can be used throughout agriculture operations. There are many more studies not covered in this short paper that demonstrate pathogen suppression, increased shelf life, and relationship with other beneficial species of life.

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