TeraGanix®

Cattle Information Packet

TeraGanix Pro™

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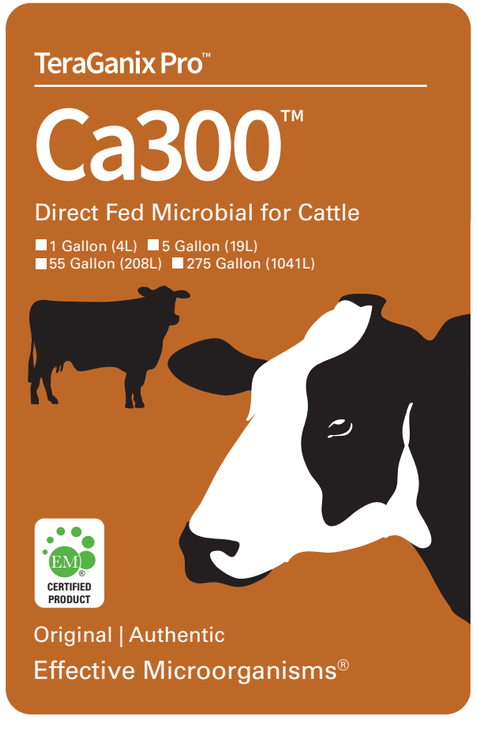
TeraGanixPro™ is our line of liquid direct feed microbial probiotics for use in livestock drinking water and as a fermentation inoculant for feeds. Ca300™ (Cattle Solution) is our liquid probiotic solution for cattle drinking water and feeds. Ca300™ uses 35 years of supporting research on Effective Microorganisms Technology® in its formulation and application. It contains only naturally-occurring live microorganisms from three genuses and all their metabolites. It is completely safe for humans, animals, and the planet. These metabolites include dozens of enzymes, trace minerals, amino acids, and vitamins that improve water quality for greater consumption, helps control pathogens by competitive exclusion, controls and reduces odors in livestock operations and dramatically enhances total digestible nutrients.



Active Ingredients:
Microorganisms: 1 million colony forming units/cc (units/ml), 1%:
Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus delbrueckii, Bacillus subtilis, Saccharomyces cerevisiae, Rhodopseudomonas palustris.

Directions for use:

Upon cattle placement:
Run Ca300™ continuously in water at a 1:2000 ratio. (In a 1:128 medicator, this will equal 1 quart of Ca300™ to 5 gallons water).

Note:
If feed passage is observed as feed rations change — run a double dose (1:1000 ratio) for 12 hrs.

Storage:
Store in a closed container. Do not store in direct sunlight. Do not allow to freeze. Store at temperatures from 50°F-90°F. Use by “Best Used” date stamped on label.

SECTION I — PRODUCT DESCRIPTION

PRODUCT NAME:	Ca300™
RECOMMENDED USES:	Cattle drinking water, water line acidifier
SYNONYMS:	Probiotic, Direct Fed Microbial
PRODUCED BY:	TeraGanix, Inc. 14193 US Highway 69 South Alto, TX 75925 (866) 369-3678 x81
CHEMICAL IDENTIFICATION:	(866) 369-3678 x81

SECTION II — HAZARD IDENTIFICATION

This section identifies the hazards of the chemical presented on the SDS and the appropriate warning information associated with those hazards.

PICTOGRAMS: None exist for Non-hazardous materials.

HAZARD RATING:

HEALTH:	0	Normal Material
FIRE:	0	Will not burn
REACTIVITY:	0	Stable

No hazardous ingredients present; not hazardous to humans, animals or plants.

GHS CLASSIFICATION: No classification exists for microorganisms.

OTHER SAFETY PRECAUTIONS: None necessary.

SECTION III — COMPOSITION/INFORMATION ON INGREDIENTS

CHEMICAL NAME	CAS#
WATER	7732-18-5
LACTIC ACID BACTERIA	Not Classified
YEAST	Not Classified
PHOTOSYNTHETIC BACTERIA	Not Classified

SECTION IV — FIRST AID MEASURES

EMERGENCY AND FIRST AID PROCEDURES

INHALATION:	In cases of inhalation, remove to freshen air.
EYES:	In case of contact with eyes, rinse with water for 10 seconds.
SKIN CONTACT:	Rinse with clean water.
INGESTION:	If swallowed, drink some fresh water.

SECTION V — FIREFIGHTING PROCEDURES

EXTINGUISHING MEDIA:	Material will not combust.
FIRE FIGHTING METHODS AND PROTECTION:	Same precautions shall be taken as for fighting a fire with water.
FIRE AND/OR EXPLOSION HAZARDS:	None
HAZARDOUS COMBUSTION PRODUCTS:	None

SECTION VI — SPILL OR LEAK PROCEDURES

STEPS TO TAKE IN CASE MATERIAL IS RELEASED OR SPILLED:

FLOORS:	Mop up with fresh water, sewer disposal.
WASTE DISPOSAL METHOD:	Sewer disposal. Material is Biodegradable, all natural organic ingredients, Non-GMO.
SOIL:	Rinse area with water.

SECTION VII — HANDLING AND STORAGE

HANDLING:	Pour material with spigot, siphon, or dosage pumps. Material digests biological matter (fats, oils, grease). Avoid contact with these materials if you don’t want them digested.
STORAGE:	Store at temperatures between (40 and 120°F). Do not freeze. Keep container closed and out of direct sunlight.
STORAGE CODE:	Green. General Chemical Storage.

SECTION VIII — PROTECTION INFORMATION

RESPIRATORY PROTECTION:	None
VENTILATION:	None
PROTECTIVE GLOVES:	Optional
EYE PROTECTION:	Safety glasses optional
PROTECTIVE CLOTHING:	None
WORK PRACTICES:	Clean up spills immediately; floor will be slick.

SECTION IX — PHYSICAL DATA

FORMULA:	Proprietary	VAPOR PRESSURE:	N/D
MOLECULAR WEIGHT:		EVAPORATION RATE:	Equal to water
APPEARANCE:	Brown liquid	VAPOR DENSITY (AIR = 1)	0.804 g/L
ODOR:	Mild	SPECIFIC GRAVITY:	1.2 (25°C)
pH:	3.5	SOLUBILITY IN WATER:	Soluble
MELTING POINT:	N/A	LOG POW (CALCULATED):	Not known
BOILING POINT:	>100°C	AUTOIGNITION TEMPERATURE:	536°C
FLASH POINT:	None	VISCOSITY:	Same as water
FLAMMABLE LIMITS IN AIR:	N/A	% VOLATILE BY VOLUME:	0

SECTION X — REACTIVITY DATA

REACTIVITY:	Not reactive under normal conditions.	HAZARDOUS DECOMPOSITION PRODUCTS:	None
CHEMICAL STABILITY:	Stable	HAZARDOUS POLYMERIZATION:	Will not occur.
INCOMPATIBLE MATERIALS:	None, non-reactive		

SECTION XI — TOXICITY DATA

ROUTES OF ENTRY: Inhalation and ingestion

SYMPTOMS (ACUTE): Coughing, abdominal discomfort (gas pain), loose stool

DISPLAYED EFFECTS: No long-term effects.

ACUTE TOXICITY:

CHEMICAL NAME	CAS NUMBER	ORAL LD50	DERMAL LD50	INHALATION LD50
WATER	7732-18-5	Oral LD50 Rat 90000 mg/kg	None Known	Not Specified
LACTIC ACID BACTERIA	Not Classified	None Known	None Known	None Known
YEAST	Not Classified	None Known	None Known	None Known
PHOTOSYNTHETIC BACTERIA	Not Classified	None Known	None Known	None Known

CARCINOGENICITY:

CHEMICAL NAME	CAS NUMBER	IARC	NTP	OSHA
WATER	7732-18-5	Not Listed	Not Listed	Not Listed
LACTIC ACID BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed
YEAST	Not Classified	Not Listed	Not Listed	Not Listed
PHOTOSYNTHETIC BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed

CHRONIC EFFECTS:

MUTAGENICITY:	No evidence of a mutagenic effect.	
TERATOGENICITY:	No evidence of a teratogenic effect (birth defect).	
SENSITIZATION:	No evidence of a sensitization effect.	
REPRODUCTIVE:	No evidence of negative reproductive effects.	
TARGET ORGAN EFFECTS:	ACUTE:	No evidence of acute effects on organs.
	CHRONIC:	No evidence of effects on the eyes.

SECTION XII — ECOLOGICAL DATA

OVERVIEW: No ecological hazards known at any concentration.

MOBILITY: This material is expected to be moderately mobile in soils. It absorbs into most soil types.

PERSISTENCE: 100% Biodegradable.

BIOACCUMULATION: Bioaccumulation is not expected to occur.

DEGRADABILITY: Biodegrades quickly.

OTHER ADVERSE EFFECTS: No Data.

CHEMICAL NAME	CAS NUMBER	ECO TOXICITY
WATER	7732-18-5	No Data Available
LACTIC ACID BACTERIA	Not Classified	No Data Available
YEAST	Not Classified	No Data Available
PHOTOSYNTHETIC BACTERIA	Not Classified	No Data Available

SECTION XIII — DISPOSAL INFORMATION

DISPOSAL METHODS: Either use old or expired product in compost or pour down a common drain.

WATER DISPOSAL CODE(S): This is an unregulated material.

SECTION XIV — TRANSPORT INFORMATION

GROUND –DOT PROPER SHIPPING NAME

AIR IATA PROPER SHIPPING NAME:

SECTION XV — REGULATORY INFORMATION

TSCA STATUS: Not on Toxic Substances Lists.

SECTION XVI — ADDITIONAL INFORMATION

REVISED 5/31/2015

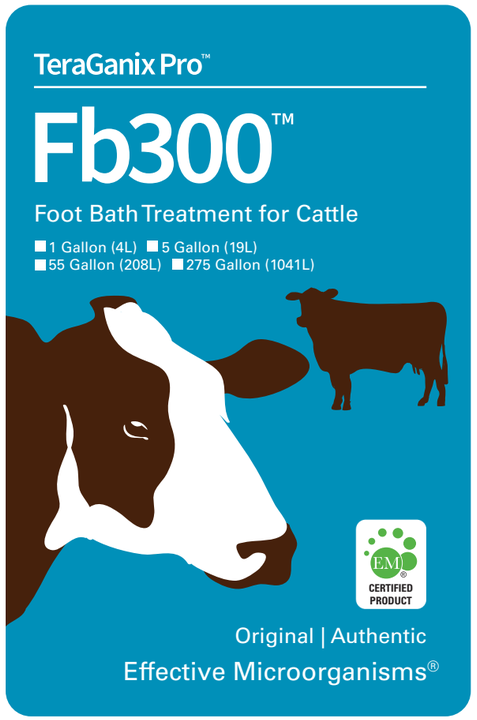
The information provided in this (Material) Safety Data Sheet represents a compilation of data drawn directly from various sources available to us. Carolina Biological Supply makes no representation or guarantee as to the suitability of this information to a particular application of the substance covered in the (Material) Safety Data Sheet.

GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
CAS	Chemical Abstract Service Number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DOT	U.S. Department of Transportation
IARC	International Agency for Research on Cancer
N/A	Not Available
IDLH	Immediately dangerous to life and health
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	Permissible Exposure Limit
ppm	Parts per million
RCRA	Resource Conservation and Recovery Act
SARA	Superfund Amendments and Reauthorization Act
TLV	Threshold Limit Value
TSCA	Toxic Substances Control Act

TeraGanix Pro™ Fb300™ Foot Bath Solution

TeraGanixPro™ is our line of liquid probiotics for use in livestock operations. Fb300™ (Foot Bath Solution) is our liquid probiotic solution for cattle foot baths. Apply Fb300™ in place of other toxic chemicals in the foot bath water. Fb300™ uses 35 years of supporting research on Effective Microorganisms Technology® in its formulation and application. It contains only naturally-occurring live microorganisms from three genera and all their metabolites. It is completely safe for humans, animals, and the planet. These metabolites include dozens of enzymes, trace minerals, amino acids, and vitamins that improve water quality, helps control pathogens by competitive exclusion, controls and reduces odors in livestock operations all without risk of negative reactions to animals or workers.



Active Ingredients:
Purified Water, Certified Organic Vinegar, Yucca Extract*, Certified Organic Grain Alcohol, Organic Sugars, EM•1® Waste Treatment*, and d-Limonene.

*OMRI Listed Ingredients.

Directions for use:

Foot Baths:
Foot bath dilutions are intended to treat 200 cows, after which it is recommended that the foot bath solution be discarded and replaced with a fresh Fb300™ foot bath solution. Animals may be walked through a foot bath of 1% (add 1/2 gallon Fb300™ to 50 gals water) to 5% (add 2.5 gals Fb300™ to 50 gals water) aqueous solution with an immersion time of 5 to 20 min twice daily for a period of time as prescribed by a veterinarian. Keep foot baths clean during treatment period. Do not allow cattle to drink from foot baths as water can be dirty. Follow instructions under Storage and Disposal when solutions are discarded at end of treatment period.

If gallon capacity of foot bath is unknown, use the following formula to calculate:

Inside Length (in.) X Inside Width (in.) X Fluid Height (in.) / 231 = gallon capacity of foot bath

Note:
To dispose, drain cleaner and recycle packaging.

Storage:
Store at room temperature in a sealed container out of direct sunlight. Best used within one year after opening.

Fb300™ SDS

SECTION I — PRODUCT DESCRIPTION

PRODUCT NAME:	Fb300™
RECOMMENDED USES:	Foot bath treatment for cattle
SYNONYMS:	Biodegradable Cleaner and Deodorizer
PRODUCED BY:	TeraGanix, Inc. 14193 US Highway 69 South Alto, TX 75925 (866) 369-3678 x81
CHEMICAL IDENTIFICATION:	(866) 369-3678 x81

SECTION II — HAZARD IDENTIFICATION

This section identifies the hazards of the chemical presented on the SDS and the appropriate warning information associated with those hazards.

PICTOGRAMS: None exist for Non-hazardous materials

HAZARD RATING:

HEALTH	0	Normal Material
FIRE	0	Will not burn
REACTIVITY	0	Stable

No hazardous ingredients present; not hazardous to humans, animals, or plants.

GHS CLASSIFICATION: No classification exists for microorganisms.

OTHER SAFETY PRECAUTIONS: None necessary.

SECTION III — COMPOSITION/INFORMATION ON INGREDIENTS

CHEMICAL NAME	CAS#
WATER	7732-18-5
LACTIC ACID BACTERIA	Not Classified
YEAST	Not Classified
PHOTOSYNTHETIC BACTERIA	Not Classified
ORGANIC GRAIN ALCOHOL	64-17-5
YUCCA SCHIDIGERA	Not Classified
d-LIMONENE	5989-27-5
ACETIC ACID (ORGANIC APPLE CIDER VINEGAR)	64-19-7
ORGANIC SUGAR	Not Classified

SECTION IV — FIRST AID MEASURES

EMERGENCY AND FIRST AID PROCEDURES

INHALATION:	In cases of inhalation, remove to freshen air.
EYES:	In case of contact with eyes, rinse with water for 10 seconds.
SKIN CONTACT:	Rinse with clean water.
INGESTION:	If swallowed, drink some fresh water.

SECTION V — FIREFIGHTING PROCEDURES

EXTINGUISHING MEDIA:	Material will not combust.
FIRE FIGHTING METHODS AND PROTECTION:	Same precautions shall be taken as for fighting a fire with water.
FIRE AND/OR EXPLOSION HAZARDS:	None
HAZARDOUS COMBUSTION PRODUCTS:	None

SECTION VI — SPILL OR LEAK PROCEDURES

STEPS TO TAKE IN CASE MATERIAL IS RELEASED OR SPILLED:

FLOORS:	Mop up with fresh water, sewer disposal.
WASTE DISPOSAL METHOD:	Sewer disposal. Material is Biodegradable, all natural organic ingredients, Non-GMO.
SOIL:	Rinse area with water.

SECTION VII — HANDLING AND STORAGE

HANDLING:	Pour material with spigot, siphon, or dosage pumps. Material digests biological matter (fats, oils, grease). Avoid contact with these materials if you don't want them digested.
STORAGE:	Store at temperatures between (40 and 120° F). Do not freeze. Keep container closed and out of direct sunlight.
STORAGE CODE:	Green. General Chemical Storage.

SECTION VIII — PROTECTION INFORMATION

RESPIRATORY PROTECTION:	None
VENTILATION:	None
PROTECTIVE GLOVES:	Optional
EYE PROTECTION:	Safety glasses optional.
PROTECTIVE CLOTHING:	None
WORK PRACTICES:	Clean up spills immediately; floor will be slick.

SECTION IX — PHYSICAL DATA

FORMULA:	Proprietary	VAPOR PRESSURE:	N/D
MOLECULAR WEIGHT:		EVAPORATION RATE:	Equal to water
APPEARANCE:	Brown liquid	VAPOR DENSITY (AIR=1)	0.804 g/L
ODOR:	Mild	SPECIFIC GRAVITY:	1.2 (25°C)
pH:	3.5	SOLUBILITY IN WATER:	Soluble
MELTING POINT:	N/A	LOG POW (CALCULATED):	Not known
BOILING POINT:	>100°C	AUTOIGNITION TEMPERATURE:	536°C
FLASH POINT:	None	VISCOSITY:	Same as water
FLAMMABLE LIMITS IN AIR:	N/A	% VOLATILE BY VOLUME:	0

SECTION X — REACTIVITY DATA

REACTIVITY:	Not reactive under normal conditions.	HAZARDOUS DECOMPOSITION PRODUCTS:	None
CHEMICAL STABILITY:	Stable	HAZARDOUS POLYMERIZATION:	Will not occur.
INCOMPATIBLE MATERIALS:	None, non-reactive		

SECTION XI — TOXICITY DATA

ROUTES OF ENTRY: Inhalation and ingestion

SYMPTOMS (ACUTE): Coughing, abdominal discomfort (gas pain), loose stool

DISPLAYED EFFECTS: No long-term effects.

ACUTE TOXICITY:

CHEMICAL NAME	CAS NUMBER	ORAL LD50	DERMAL LD50	INHALATION LD50
WATER	7732-18-5	Oral LD50 Rat 90000 mg/kg	None Known	Not Specified
LACTIC ACID BACTERIA	Not Classified	None Known	None Known	None Known
YEAST	Not Classified	None Known	None Known	None Known
PHOTOSYNTHETIC BACTERIA	Not Classified	None Known	None Known	None Known
ORGANIC GRAIN ALCOHOL	64-17-5	50mg/kg	May cause redness	Slightly Toxic
YUCCA SCHIDIGERA	223749-05-1	Human: 5000mg/kg	No	No
d-LIMONENE	94266-47-4	Rat: 2000mg/kg Rabbit: 5000mg/kg		
ACETIC ACID (ORGANIC APPLE CIDER VINEGAR)	64-19-7	Not Classified	Not Classified	Not Classified
ORGANIC SUGAR	8052-35-5	Not Classified	Not Classified	Not Classified

CARCINOGENICITY:

CHEMICAL NAME	CAS NUMBER	IARC	NTP	OSHA
WATER	7732-18-5	Not Listed	Not Listed	Not Listed
LACTIC ACID BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed
YEAST	Not Classified	Not Listed	Not Listed	Not Listed
PHOTOSYNTHETIC BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed
ORGANIC GRAIN ALCOHOL	64-17-5	Not Classified	Not Classified	Not Classified
YUCCA SCHIDIGERA	223749-05-1	Not Classified	Not Classified	Not Classified
d-LIMONENE	94266-47-4	Not Classified	Not Classified	Not Classified
ACETIC ACID (ORGANIC APPLE CIDER VINEGAR)	64-19-7	No	No	No
ORGANIC SUGAR	8052-35-5	No	No	No

CHRONIC EFFECTS

MUTAGENICITY:	No evidence of a mutagenic effect.	
TERATOGENICITY:	No evidence of a teratogenic effect (birth defect).	
SENSITIZATION:	No evidence of a sensitization effect.	
REPRODUCTIVE:	No evidence of negative reproductive effects.	
TARGET ORGAN EFFECTS:	Acute:	No evidence of acute effects on organs.
	Chronic:	No evidence of effects on the eyes.

SECTION XII — ECOLOGICAL DATA

OVERVIEW:	No ecological hazards known at any concentration.
MOBILITY:	This material is expected to be moderately mobile in soils. It absorbs into most soil types.
PERSISTENCE:	Finished product in container is 100% Biodegradable.
BIOACCUMULATION:	Bioaccumulation is not expected to occur.
DEGRADABILITY:	Biodegrades quickly.
OTHER ADVERSE EFFECTS:	No Data.

CHEMICAL NAME	CAS NUMBER	ECO TOXICITY
WATER	7732-18-5	No Data Available
LACTIC ACID BACTERIA	Not Classified	No Data Available
YEAST	Not Classified	No Data Available
PHOTOSYNTHETIC BACTERIA	Not Classified	No Data Available
ORGANIC GRAIN ALCOHOL	64-17-5	Highly toxic to aquatic life
YUCCA SCHIDIGERA	223749-05-1	21 CFR 172.510 Considered non-toxic
d-LIMONENE	94266-47-4	May be toxic to fish
ACETIC ACID (ORGANIC APPLE CIDER VINEGAR)	64-19-7	No Data Available
ORGANIC SUGAR	8052-35-5	None known

SECTION XIII — DISPOSAL INFORMATION

DISPOSAL METHODS:	Either use old or expired product in compost or pour down a common drain.
WATER DISPOSAL CODE(S):	This is an unregulated material.

SECTION XIV — TRANSPORT INFORMATION

GROUND –DOT PROPER SHIPPING NAME

AIR IATA Proper Shipping Name:

SECTION XV — REGULATORY INFORMATION

TSCA STATUS: Not on Toxic Substances Lists

SECTION XVI — ADDITIONAL INFORMATION

REVISED 5/31/2015

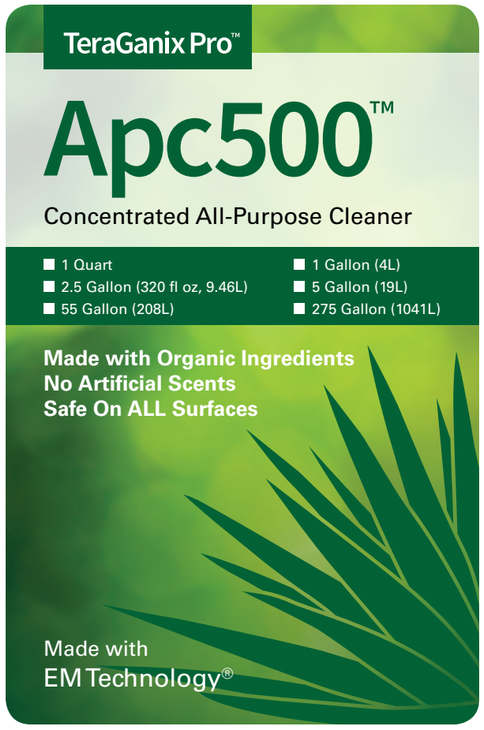
The information provided in this (Material) Safety Data Sheet represents a compilation of data drawn directly from various sources available to us. Carolina Biological Supply makes no representation or guarantee as to the suitability of this information to a particular application of the substance covered in the (Material) Safety Data Sheet.

GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
CAS	Chemical Abstract Service Number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DOT	U.S. Department of Transportation
IARC	International Agency for Research on Cancer
N/A	Not Available
IDLH	Immediately dangerous to life and health
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	Permissible Exposure Limit
ppm	Parts per million
RCRA	Resource Conservation and Recovery Act
SARA	Superfund Amendments and Reauthorization Act
TLV	Threshold Limit Value
TSCA	Toxic Substances Control Act

TeraGanix Pro™ Apc500™ All-Purpose Cleaner

TeraGanixPro™ is our line of liquid probiotics for use in livestock operations. Apc500™ (All Purpose Cleaner) is our liquid probiotic cleaning solution for cleaning all surfaces including metals, concrete, equipment, and facilities. Use Apc500™ for cleaning flooring, walls, vehicles, and other equipment around your livestock operation. Apc500™ uses 35 years of supporting research on Effective Microorganisms Technology® in its formulation and application. This non-corrosive formula is made of all food grade ingredients and cleans and deodorizes without the use of harsh chemicals or perfumes (most of which are classified as carcinogens). It is completely safe for humans, animals, and the planet. Our cost-effective formula is made with all food-grade, natural, and certified organic ingredients.



Active Ingredients:
Purified Water, Certified Organic Vinegar, Yucca Extract*, Certified Organic Grain Alcohol, Organic Sugars, EM•1® Waste Treatment*, and d-Limonene.

*OMRI Listed Ingredients.

Directions for use:
Dilute Apc500™ with clean water. Fill a trigger spray bottle and spray surface until visibly wet. Soak area if desired. Wait 1–10 minutes and wipe dry with squeegee or microfiber cloth. Repeat as necessary.

Note:
For best results, use microfiber cloths when cleaning. Keep this and all cleaning products out of reach of children. Test on delicate surfaces for dilution rates.
To dispose, drain cleaner and recycle packaging.

Storage:
Store at room temperature in a sealed container out of direct sunlight. Best used within one year after opening.

Apc500™ SDS

SECTION I — PRODUCT DESCRIPTION

PRODUCT NAME:	TeraGanix Pro Apc500™
RECOMMENDED USES:	All Purpose Cleaning, degreasing, deodorizing
SYNONYMS:	Biodegradable Cleaner and Deodorizer
PRODUCED BY:	TeraGanix, Inc. 14193 US Highway 69 South Alto, TX 75925 (866) 369-3678 x81
CHEMICAL IDENTIFICATION:	(866) 369-3678 x81

SECTION II — HAZARD IDENTIFICATION

This section identifies the hazards of the chemical presented on the SDS and the appropriate warning information associated with those hazards.

PICTOGRAMS: None exist for Non-hazardous materials

HAZARD RATING:

HEALTH	0	Normal Material
FIRE	0	Will not burn
REACTIVITY	0	Stable

No hazardous ingredients present; not hazardous to humans, animals or plants.

GHS CLASSIFICATION: No classification exists for microorganisms.

OTHER SAFETY PRECAUTIONS: None necessary.

SECTION III — COMPOSITION/INFORMATION ON INGREDIENTS

CHEMICAL NAME	CAS#
WATER	7732-18-5
LACTIC ACID BACTERIA	Not Classified
YEAST	Not Classified
PHOTOSYNTHETIC BACTERIA	Not Classified
ORGANIC GRAIN ALCOHOL	64-17-5
YUCCA SCHIDIGERA	Not Classified
d-LIMONENE	5989-27-5
ACETIC ACID (ORGANIC APPLE CIDER VINEGAR)	64-19-7
ORGANIC SUGAR	Not Classified

SECTION IV — FIRST AID MEASURES

EMERGENCY AND FIRST AID PROCEDURES

INHALATION:	In cases of inhalation, remove to freshen air.
EYES:	In case of contact with eyes, rinse with water for 10 seconds.
SKIN CONTACT:	Rinse with clean water.
INGESTION:	If swallowed, drink some fresh water.

SECTION V — FIREFIGHTING PROCEDURES

EXTINGUISHING MEDIA:	Material will not combust.
FIRE FIGHTING METHODS AND PROTECTION:	Same precautions shall be taken as for fighting a fire with water.
FIRE AND/OR EXPLOSION HAZARDS:	None
HAZARDOUS COMBUSTION PRODUCTS:	None

SECTION VI — SPILL OR LEAK PROCEDURES

STEPS TO TAKE IN CASE MATERIAL IS RELEASED OR SPILLED:

FLOORS:	Mop up with fresh water, sewer disposal.
WASTE DISPOSAL METHOD:	Sewer disposal. Material is Biodegradable, all natural organic ingredients, Non-GMO.
SOIL:	Rinse area with water.

SECTION VII — HANDLING AND STORAGE

HANDLING:	Pour material with spigot, siphon, or dosage pumps. Material digests biological matter (fats, oils, grease). Avoid contact with these materials if you don't want them digested.
STORAGE:	Store at temperatures between (40 and 120° F). Do not freeze. Keep container closed and out of direct sunlight.
STORAGE CODE:	Green. General Chemical Storage.

SECTION VIII — PROTECTION INFORMATION

RESPIRATORY PROTECTION:	None
VENTILATION:	None
PROTECTIVE GLOVES:	Optional
EYE PROTECTION:	Safety glasses optional.
PROTECTIVE CLOTHING:	None
WORK PRACTICES:	Clean up spills immediately; floor will be slick.

SECTION IX — PHYSICAL DATA

FORMULA:	Proprietary	VAPOR PRESSURE:	N/D
MOLECULAR WEIGHT:		EVAPORATION RATE:	Equal to water
APPEARANCE:	Brown liquid	VAPOR DENSITY (AIR=1)	0.804 g/L
ODOR:	Mild	SPECIFIC GRAVITY:	1.2 (25°C)
pH:	3.5	SOLUBILITY IN WATER:	Soluble
MELTING POINT:	N/A	LOG POW (CALCULATED):	Not known
BOILING POINT:	>100°C	AUTOIGNITION TEMPERATURE:	536°C
FLASH POINT:	None	VISCOSITY:	Same as water
FLAMMABLE LIMITS IN AIR:	N/A	% VOLATILE BY VOLUME:	0

SECTION X — REACTIVITY DATA

REACTIVITY:	Not reactive under normal conditions.	HAZARDOUS DECOMPOSITION PRODUCTS:	None
CHEMICAL STABILITY:	Stable	HAZARDOUS POLYMERIZATION:	Will not occur.
INCOMPATIBLE MATERIALS:	None, non-reactive		

SECTION XI — TOXICITY DATA

ROUTES OF ENTRY: Inhalation and ingestion

SYMPTOMS (ACUTE): Coughing, abdominal discomfort (gas pain), loose stool

DISPLAYED EFFECTS: No long-term effects.

ACUTE TOXICITY:

CHEMICAL NAME	CAS NUMBER	ORAL LD50	DERMAL LD50	INHALATION LD50
WATER	7732-18-5	Oral LD50 Rat 90000 mg/kg	None Known	Not Specified
LACTIC ACID BACTERIA	Not Classified	None Known	None Known	None Known
YEAST	Not Classified	None Known	None Known	None Known
PHOTOSYNTHETIC BACTERIA	Not Classified	None Known	None Known	None Known
ORGANIC GRAIN ALCOHOL	64-17-5	50mg/kg	May cause redness	Slightly Toxic
YUCCA SCHIDIGERA	223749-05-1	Human: 5000mg/kg	No	No
d-LIMONENE	94266-47-4	Rat: 2000mg/kg Rabbit: 5000mg/kg		
ACETIC ACID (ORGANIC APPLE CIDER VINEGAR)	64-19-7	Not Classified	Not Classified	Not Classified
ORGANIC SUGAR	8052-35-5	Not Classified	Not Classified	Not Classified

CARCINOGENICITY:

CHEMICAL NAME	CAS NUMBER	IARC	NTP	OSHA
WATER	7732-18-5	Not Listed	Not Listed	Not Listed
LACTIC ACID BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed
YEAST	Not Classified	Not Listed	Not Listed	Not Listed
PHOTOSYNTHETIC BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed
ORGANIC GRAIN ALCOHOL	64-17-5	Not Classified	Not Classified	Not Classified
YUCCA SCHIDIGERA	223749-05-1	Not Classified	Not Classified	Not Classified
d-LIMONENE	94266-47-4	Not Classified	Not Classified	Not Classified
ACETIC ACID (ORGANIC APPLE CIDER VINEGAR)	64-19-7	No	No	No
ORGANIC SUGAR	8052-35-5	No	No	No

CHRONIC EFFECTS

MUTAGENICITY:	No evidence of a mutagenic effect.	
TERATOGENICITY:	No evidence of a teratogenic effect (birth defect).	
SENSITIZATION:	No evidence of a sensitization effect.	
REPRODUCTIVE:	No evidence of negative reproductive effects.	
TARGET ORGAN EFFECTS:	Acute:	No evidence of acute effects on organs.
	Chronic:	No evidence of effects on the eyes.

SECTION XII — ECOLOGICAL DATA

OVERVIEW:	No ecological hazards known at any concentration.
MOBILITY:	This material is expected to be moderately mobile in soils. It absorbs into most soil types.
PERSISTENCE:	Finished product in container is 100% Biodegradable.
BIOACCUMULATION:	Bioaccumulation is not expected to occur.
DEGRADABILITY:	Biodegrades quickly.
OTHER ADVERSE EFFECTS:	No Data.

CHEMICAL NAME	CAS NUMBER	ECO TOXICITY
WATER	7732-18-5	No Data Available
LACTIC ACID BACTERIA	Not Classified	No Data Available
YEAST	Not Classified	No Data Available
PHOTOSYNTHETIC BACTERIA	Not Classified	No Data Available
ORGANIC GRAIN ALCOHOL	64-17-5	Highly toxic to aquatic life
YUCCA SCHIDIGERA	223749-05-1	21 CFR 172.510 Considered non-toxic
d-LIMONENE	94266-47-4	May be toxic to fish
ACETIC ACID (ORGANIC APPLE CIDER VINEGAR)	64-19-7	No Data Available
ORGANIC SUGAR	8052-35-5	None known

SECTION XIII — DISPOSAL INFORMATION

DISPOSAL METHODS:	Either use old or expired product in compost or pour down a common drain.
WATER DISPOSAL CODE(S):	This is an unregulated material.

SECTION XIV — TRANSPORT INFORMATION

GROUND –DOT PROPER SHIPPING NAME

AIR IATA Proper Shipping Name:

SECTION XV — REGULATORY INFORMATION

TSCA STATUS: Not on Toxic Substances Lists

SECTION XVI — ADDITIONAL INFORMATION

REVISED 5/31/2015

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GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
CAS	Chemical Abstract Service Number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DOT	U.S. Department of Transportation
IARC	International Agency for Research on Cancer
N/A	Not Available
IDLH	Immediately dangerous to life and health
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	Permissible Exposure Limit
ppm	Parts per million
RCRA	Resource Conservation and Recovery Act
SARA	Superfund Amendments and Reauthorization Act
TLV	Threshold Limit Value
TSCA	Toxic Substances Control Act

TeraGanixPro™ is our line of liquid probiotics for use in livestock operations. Wt1000™ (Waste Treatment Solution) is our liquid probiotic solution for odor control and digestion of solids. Wt1000™ uses 35 years of supporting research on Effective Microorganisms Technology® in its formulation and application. Since Wt1000™ is perfectly safe around animals, this all-natural probiotic formula can be used in misters for odor control or even sprayed directly onto manure inside barns and outdoors. In lagoons it greatly reduces solids, digesting sludge, increasing the life of the lagoon. It can be used in flushing systems to clean runways, eliminating cleanup times and dramatically reduce slippage, all without the worry of corroding concrete or metals.

Active Ingredients:
Microorganisms: 1 million colony forming units/cc (units/ml) 1%: Lactobacillus casei, Rhodopseudomonas palustris, Saccharomyces cerevisiae.

Directions for use:
For environmental applications.
Livestock odors 2–3 times per week
1:64 (2oz / Gal).

Note:
White Flakes may appear on the surface due to live cultures. Sediment at bottom of bottle is natural.

Storage:
Do not store Wt1000™ in direct sunlight. Store in closed container at room temperature. Do not freeze.

SECTION I — PRODUCT DESCRIPTION

PRODUCT NAME:	Wt1000™
RECOMMENDED USES:	Wastewater Treatment, Livestock Litter Treatment, Odor Control
SYNONYMS:	Wastewater Treatment, Deordorizers
PRODUCED BY:	TeraGanix, Inc. 14193 US Highway 69 South Alto, TX 75925 (866) 369-3678 x81
CHEMICAL IDENTIFICATION:	(866) 369-3678 x81

SECTION II — HAZARD IDENTIFICATION

This section identifies the hazards of the chemical presented on the SDS and the appropriate warning information associated with those hazards.

PICTOGRAMS: None exist for Non-hazardous materials.

HAZARD RATING:

HEALTH:	0	Normal Material
FIRE:	0	Will not burn
REACTIVITY:	0	Stable

No hazardous ingredients present; not hazardous to humans, animals or plants.

GHS CLASSIFICATION: No classification exists for microorganisms.

OTHER SAFETY PRECAUTIONS: None necessary.

SECTION III — COMPOSITION/INFORMATION ON INGREDIENTS

CHEMICAL NAME	CAS#
WATER	7732-18-5
LACTIC ACID BACTERIA	Not Classified
YEAST	Not Classified
PHOTOSYNTHETIC BACTERIA	Not Classified

SECTION IV — FIRST AID MEASURES

EMERGENCY AND FIRST AID PROCEDURES

INHALATION:	In cases of inhalation, remove to freshen air.
EYES:	In case of contact with eyes, rinse with water for 10 seconds.
SKIN CONTACT:	Rinse with clean water.
INGESTION:	If swallowed, drink some fresh water.

SECTION V — FIREFIGHTING PROCEDURES

EXTINGUISHING MEDIA:	Material will not combust.
FIRE FIGHTING METHODS AND PROTECTION:	Same precautions shall be taken as for fighting a fire with water.
FIRE AND/OR EXPLOSION HAZARDS:	None
HAZARDOUS COMBUSTION PRODUCTS:	None

SECTION VI — SPILL OR LEAK PROCEDURES

STEPS TO TAKE IN CASE MATERIAL IS RELEASED OR SPILLED:

FLOORS:	Mop up with fresh water, sewer disposal.
WASTE DISPOSAL METHOD:	Sewer disposal. Material is Biodegradable, all natural organic ingredients, Non-GMO.
SOIL:	Rinse area with water.

SECTION VII — HANDLING AND STORAGE

HANDLING:	Pour material with spigot, siphon, or dosage pumps. Material digests biological matter (fats, oils, grease). Avoid contact with these materials if you don't want them digested.
STORAGE:	Store at temperatures between (40 and 120°F). Do not freeze. Keep container closed and out of direct sunlight.
STORAGE CODE:	Green. General Chemical Storage.

SECTION VIII — PROTECTION INFORMATION

RESPIRATORY PROTECTION:	None
VENTILATION:	None
PROTECTIVE GLOVES:	Optional
EYE PROTECTION:	Safety glasses optional
PROTECTIVE CLOTHING:	None
WORK PRACTICES:	Clean up spills immediately; floor will be slick.

SECTION IX — PHYSICAL DATA

FORMULA:	Proprietary	VAPOR PRESSURE:	N/D
MOLECULAR WEIGHT:		EVAPORATION RATE:	Equal to water
APPEARANCE:	Brown liquid	VAPOR DENSITY (AIR = 1)	0.804 g/L
ODOR:	Mild	SPECIFIC GRAVITY:	1.2 (25°C)
pH:	3.5	SOLUBILITY IN WATER:	Soluble
MELTING POINT:	N/A	LOG POW (CALCULATED):	Not known
BOILING POINT:	>100°C	AUTOIGNITION TEMPERATURE:	536°C
FLASH POINT:	None	VISCOSITY:	Same as water
FLAMMABLE LIMITS IN AIR:	N/A	% VOLATILE BY VOLUME:	0

SECTION X — REACTIVITY DATA

REACTIVITY:	Not reactive under normal conditions.	HAZARDOUS DECOMPOSITION PRODUCTS:	None
CHEMICAL STABILITY:	Stable	HAZARDOUS POLYMERIZATION:	Will not occur.
INCOMPATIBLE MATERIALS:	None, non-reactive		

SECTION XI — TOXICITY DATA

ROUTES OF ENTRY: Inhalation and ingestion

SYMPTOMS (ACUTE): Coughing, abdominal discomfort (gas pain), loose stool

DISPLAYED EFFECTS: No long-term effects.

ACUTE TOXICITY:

CHEMICAL NAME	CAS NUMBER	ORAL LD50	DERMAL LD50	INHALATION LD50
WATER	7732-18-5	Oral LD50 Rat 90000 mg/kg	None Known	Not Specified
LACTIC ACID BACTERIA	Not Classified	None Known	None Known	None Known
YEAST	Not Classified	None Known	None Known	None Known
PHOTOSYNTHETIC BACTERIA	Not Classified	None Known	None Known	None Known

CARCINOGENICITY:

CHEMICAL NAME	CAS NUMBER	IARC	NTP	OSHA
WATER	7732-18-5	Not Listed	Not Listed	Not Listed
LACTIC ACID BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed
YEAST	Not Classified	Not Listed	Not Listed	Not Listed
PHOTOSYNTHETIC BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed

CHRONIC EFFECTS:

MUTAGENICITY:	No evidence of a mutagenic effect.	
TERATOGENICITY:	No evidence of a teratogenic effect (birth defect).	
SENSITIZATION:	No evidence of a sensitization effect.	
REPRODUCTIVE:	No evidence of negative reproductive effects.	
TARGET ORGAN EFFECTS:	ACUTE:	No evidence of acute effects on organs.
	CHRONIC:	No evidence of effects on the eyes.

SECTION XII — ECOLOGICAL DATA

OVERVIEW: No ecological hazards known at any concentration.

MOBILITY: This material is expected to be moderately mobile in soils. It absorbs into most soil types.

PERSISTENCE: 100% Biodegradable.

BIOACCUMULATION: Bioaccumulation is not expected to occur.

DEGRADABILITY: Biodegrades quickly.

OTHER ADVERSE EFFECTS: No Data.

CHEMICAL NAME	CAS NUMBER	ECO TOXICITY
WATER	7732-18-5	No Data Available
LACTIC ACID BACTERIA	Not Classified	No Data Available
YEAST	Not Classified	No Data Available
PHOTOSYNTHETIC BACTERIA	Not Classified	No Data Available

SECTION XIII — DISPOSAL INFORMATION

DISPOSAL METHODS: Either use old or expired product in compost or pour down a common drain.

WATER DISPOSAL CODE(S): This is an unregulated material.

SECTION XIV — TRANSPORT INFORMATION

GROUND –DOT PROPER SHIPPING NAME

AIR IATA PROPER SHIPPING NAME:

SECTION XV — REGULATORY INFORMATION

TSCA STATUS: Not on Toxic Substances Lists.

SECTION XVI — ADDITIONAL INFORMATION

REVISED 5/31/2015

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GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
CAS	Chemical Abstract Service Number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DOT	U.S. Department of Transportation
IARC	International Agency for Research on Cancer
N/A	Not Available
IDLH	Immediately dangerous to life and health
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	Permissible Exposure Limit
ppm	Parts per million
RCRA	Resource Conservation and Recovery Act
SARA	Superfund Amendments and Reauthorization Act
TLV	Threshold Limit Value
TSCA	Toxic Substances Control Act

TeraGanix Pro™
Ag1000® Agricultural Concentrate

TeraGanixPro™ is our line of liquid probiotics for use in livestock operations. Ag1000® (Agricultural Solution) is our liquid probiotic solution for growing crops and for composting manures. Ag1000® uses 35 years of supporting research on Effective Microorganisms Technology® in its formulation and application. Ag1000® improves soil tilth and increases nutrient efficiency while decreasing negative impacts of salts. Used in composting it increases airflow that results in higher populations of aerobic microbes, including several fungal species. This makes for a faster finished compost that does not require any curing before use.
We have documented biological maturity and stability at 45 days. This means you can completely eliminate the 90+ day curing time required with regular composting. Ag1000® Organic is an OMRI Listed version for certified organic operations.

Active Ingredients:
Microorganisms: 1 million colony forming units/cc (units/ml) 1%: Lactobacillus casei.

Directions for use:
Apply to soils using irrigation, pre-plant incorporated, or pre-emerge, at a rate of 10 to 45 gallons per acre.

Note:
White flakes may appear on the surface due to live cultures. Sediment at bottom of container is natural.

Storage:
Do not store Ag1000® in direct sunlight. Store in closed container between 40°F and 100°F. Do not freeze.

Ag1000® SDS

SECTION I — PRODUCT DESCRIPTION

PRODUCT NAME:	Ag1000®
RECOMMENDED USES:	For all soils and plants as part of a crop nutrient program
SYNONYMS:	Composting, Deordorizers
PRODUCED BY:	TeraGanix, Inc. 14193 US Highway 69 South Alto, TX 75925 (866) 369-3678 x81
CHEMICAL IDENTIFICATION:	(866) 369-3678 x81

SECTION II — HAZARD IDENTIFICATION

This section identifies the hazards of the chemical presented on the SDS and the appropriate warning information associated with those hazards.

PICTOGRAMS: None exist for Non-hazardous materials.

HAZARD RATING:

HEALTH:	0	Normal Material
FIRE:	0	Will not burn
REACTIVITY:	0	Stable

No hazardous ingredients present; not hazardous to humans, animals or plants.

GHS CLASSIFICATION: No classification exists for microorganisms.

OTHER SAFETY PRECAUTIONS: None necessary.

SECTION III — COMPOSITION/INFORMATION ON INGREDIENTS

CHEMICAL NAME	CAS#
WATER	7732-18-5
LACTIC ACID BACTERIA	Not Classified
YEAST	Not Classified
PHOTOSYNTHETIC BACTERIA	Not Classified

SECTION IV — FIRST AID MEASURES

EMERGENCY AND FIRST AID PROCEDURES

INHALATION:	In cases of inhalation, remove to freshen air.
EYES:	In case of contact with eyes, rinse with water for 10 seconds.
SKIN CONTACT:	Rinse with clean water.
INGESTION:	If swallowed, drink some fresh water.

SECTION V — FIREFIGHTING PROCEDURES

EXTINGUISHING MEDIA:	Material will not combust.
FIRE FIGHTING METHODS AND PROTECTION:	Same precautions shall be taken as for fighting a fire with water.
FIRE AND/OR EXPLOSION HAZARDS:	None
HAZARDOUS COMBUSTION PRODUCTS:	None

SECTION VI — SPILL OR LEAK PROCEDURES

STEPS TO TAKE IN CASE MATERIAL IS RELEASED OR SPILLED:

FLOORS:	Mop up with fresh water, sewer disposal.
WASTE DISPOSAL METHOD:	Sewer disposal. Material is Biodegradable, all natural organic ingredients, Non-GMO.
SOIL:	Rinse area with water.

SECTION VII — HANDLING AND STORAGE

HANDLING:	Pour material with spigot, siphon, or dosage pumps. Material digests biological matter (fats, oils, grease). Avoid contact with these materials if you don't want them digested.
STORAGE:	Store at temperatures between (40 and 120°F). Do not freeze. Keep container closed and out of direct sunlight.
STORAGE CODE:	Green. General Chemical Storage.

SECTION VIII — PROTECTION INFORMATION

RESPIRATORY PROTECTION:	None
VENTILATION:	None
PROTECTIVE GLOVES:	Optional
EYE PROTECTION:	Safety glasses optional
PROTECTIVE CLOTHING:	None
WORK PRACTICES:	Clean up spills immediately; floor will be slick.

SECTION IX — PHYSICAL DATA

FORMULA:	Proprietary	VAPOR PRESSURE:	N/D
MOLECULAR WEIGHT:		EVAPORATION RATE:	Equal to water
APPEARANCE:	Brown liquid	VAPOR DENSITY (AIR = 1)	0.804 g/L
ODOR:	Mild	SPECIFIC GRAVITY:	1.2 (25°C)
pH:	3.5	SOLUBILITY IN WATER:	Soluble
MELTING POINT:	N/A	LOG POW (CALCULATED):	Not known
BOILING POINT:	>100°C	AUTOIGNITION TEMPERATURE:	536°C
FLASH POINT:	None	VISCOSITY:	Same as water
FLAMMABLE LIMITS IN AIR:	N/A	% VOLATILE BY VOLUME:	0

SECTION X — REACTIVITY DATA

REACTIVITY:	Not reactive under normal conditions.	HAZARDOUS DECOMPOSITION PRODUCTS:	None
CHEMICAL STABILITY:	Stable	HAZARDOUS POLYMERIZATION:	Will not occur.
INCOMPATIBLE MATERIALS:	None, non-reactive		

SECTION XI — TOXICITY DATA

ROUTES OF ENTRY: Inhalation and ingestion

SYMPTOMS (ACUTE): Coughing, abdominal discomfort (gas pain), loose stool

DISPLAYED EFFECTS: No long-term effects.

ACUTE TOXICITY:

CHEMICAL NAME	CAS NUMBER	ORAL LD50	DERMAL LD50	INHALATION LD50
WATER	7732-18-5	Oral LD50 Rat 90000 mg/kg	None Known	Not Specified
LACTIC ACID BACTERIA	Not Classified	None Known	None Known	None Known
YEAST	Not Classified	None Known	None Known	None Known
PHOTOSYNTHETIC BACTERIA	Not Classified	None Known	None Known	None Known

CARCINOGENICITY:

CHEMICAL NAME	CAS NUMBER	IARC	NTP	OSHA
WATER	7732-18-5	Not Listed	Not Listed	Not Listed
LACTIC ACID BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed
YEAST	Not Classified	Not Listed	Not Listed	Not Listed
PHOTOSYNTHETIC BACTERIA	Not Classified	Not Listed	Not Listed	Not Listed

CHRONIC EFFECTS:

MUTAGENICITY:	No evidence of a mutagenic effect.	
TERATOGENICITY:	No evidence of a teratogenic effect (birth defect).	
SENSITIZATION:	No evidence of a sensitization effect.	
REPRODUCTIVE:	No evidence of negative reproductive effects.	
TARGET ORGAN EFFECTS:	ACUTE:	No evidence of acute effects on organs.
	CHRONIC:	No evidence of effects on the eyes.

SECTION XII — ECOLOGICAL DATA

OVERVIEW: No ecological hazards known at any concentration.

MOBILITY: This material is expected to be moderately mobile in soils. It absorbs into most soil types.

PERSISTENCE: 100% Biodegradable.

BIOACCUMULATION: Bioaccumulation is not expected to occur.

DEGRADABILITY: Biodegrades quickly.

OTHER ADVERSE EFFECTS: No Data.

CHEMICAL NAME	CAS NUMBER	ECO TOXICITY
WATER	7732-18-5	No Data Available
LACTIC ACID BACTERIA	Not Classified	No Data Available
YEAST	Not Classified	No Data Available
PHOTOSYNTHETIC BACTERIA	Not Classified	No Data Available

SECTION XIII — DISPOSAL INFORMATION

DISPOSAL METHODS: Either use old or expired product in compost or pour down a common drain.

WATER DISPOSAL CODE(S): This is an unregulated material.

SECTION XIV — TRANSPORT INFORMATION

GROUND –DOT PROPER SHIPPING NAME

AIR IATA PROPER SHIPPING NAME:

SECTION XV — REGULATORY INFORMATION

TSCA STATUS: Not on Toxic Substances Lists.

SECTION XVI — ADDITIONAL INFORMATION

REVISED 5/31/2015

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GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
CAS	Chemical Abstract Service Number
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DOT	U.S. Department of Transportation
IARC	International Agency for Research on Cancer
N/A	Not Available
IDLH	Immediately dangerous to life and health
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	Permissible Exposure Limit
ppm	Parts per million
RCRA	Resource Conservation and Recovery Act
SARA	Superfund Amendments and Reauthorization Act
TLV	Threshold Limit Value
TSCA	Toxic Substances Control Act

Study #1

EM Effects of Preventing *Staphylococcus Aureus* from Propagation

October 1995,
Minako Matsumori

OBJECTIVE

Mastitis is the increase in the number of debilitating and disease bearing cells within udder milk, affecting the qualities of the milk. The financial impact of each case of mastitis has been calculated at some 50,000 yen. The primary pathogenic bacteria in mastitis is *Staphylococcus aureus*, which is involved in 60% of all cases, while the streptococcus accounts for about 30% of the total. The remaining 10% appears to be caused by corynebacterium, coliform bacteria, and fungus.

A primary characteristic of the main culprit, *Staphylococcus aureus*, is the appearance of growths in the mammary gland, which, once infected, is difficult to treat and quickly becomes a chronic problem. Therefore, this study reviewed preventing or suppressing the growth of *Staphylococcus aureus* through the use of Effective Microorganisms® (EM).

MATERIALS AND METHODS

MATERIALS

- Milk: Taken on 16 October 1995 from cows infected with mastitis.
- Kyusei EM1: Manufactured by the Products Department of the International Nature Farming Research Center.
  Main microorganisms include: lactic acid bacteria, photosynthetic bacteria, yeast fungi, filamentous fungi, and actinomycetes.
  Manufactured on September 6, 1995.
- Molasses: Beet molasses.
- Mannitol Salt Agar with Egg Yolk:
  (Meat extract, pepton, sodium chloride, mannitol, phenol red, agar, and egg yolk.)
- Mannitol Salt Liquid Medium:
  (Meat extract, pepton, and mannitol.)
- PS Latex "Eiken": Test for coagulase production, and will be used in identifying *Staphylococcus aureus*. Coaglase is a special enzyme produced by *Staphylococcus aureus*. PS Latex is floating in buffer and sensitized with human plasma.
- Rabbit Plasma "Eiken": Test for coagulase production, and will be used in identifying *Staphylococcus aureus*. It is consisted from frozen 1 ml of rabbit plasma.

METHODS

The Isolation and Identification of *Staphylococcus aureus* and its Cultivation.

The milk is diluted in sterilized saline solution in seven steps from 10^{-1} to 10^{-7}. The diluted liquid is inoculated into the mannitol salt agar with egg yolk, then cultivated for approximately 43 hours at 37°C. Colonies which grow by incubation are analyzed and identified *Staphylococcus aureus* using PS latex.

The identified *Staphylococcus aureus* is cultivated for approximately 24 hours at 37°C in a mannitol salt liquid medium.

a. **Directions for the Mannitol Salt Agar with Egg Yolk**

   Dissolve the mannitol salt agar in distilled water, place into autoclave to undergo high pressure sterilization. Add 10 ml of a septically removed egg yolk. (Egg yolk is added because the culture will become cloudy as a result of the *Staphylococcus aureus* reacting with the yolk.) The completed solution is poured into sterilized Petri-dishes.

b. **Directions for Making the Mannitol Salt Liquid Medium**

   Dissolve the mannitol, pepton, and meat extract into distilled water, place into autoclave to undergo high pressure sterilization.

c. **Usage of Rabbit Plasma "Eiken"**

   Add 7 ml of sterile physiological saline solution to dried plasma, and dissolve. Divide 0.5 ml of this plasma solution into a test tube. Concurrently, the amount of one platinum loop of cultured analyte in the agar medium for one night are portioned into the test tubes mentioned above, then mixed with the plasma solution. It is cultured for three hours at 37°C, and examined. It is deemed positive if the plasma solution is coagulated.

d. **Usage of +PS Latex**

   The analyte is smeared on a slide and mixed with physiological saline solution. Then the PS latex solution is dropped on it, and it is agitated gently for one minute. If cohesion takes place within one minute, it is considered positive, while if cohesion does not take place, it is negative.

Cultivation of EM and the Growth Inhibition Test of *Staphylococcus aureus* by EM.

This culture is diluted with a sterilized physiological saline solution in eight steps from 10^{-1} to 10^{-8}.

These diluted solutions are inoculated onto the mannitol salt egg yolk agar and are then cultured at 37°C for approximately 20 hours. The colonies formed on the culture are counted, and then the number of bacteria of the culture that is diluted by 10^{-4} is calculated. This culture, diluted by 10^{-4}, is called A.

1.8 ml of EM is stirred in with 9 ml of sterilized molasses diluted 50 times (1% pepton, 0.1% meat extract are added) and cultured for one day at 37°C. This liquid culture is called B.

The mixture of one ml of A and one ml of B, and eight ml of the 50 times dilution of sterilized molasses (1% pepton, 0.1% meat extract are added) is called No. 1. In addition, a mixture of one ml of A and nine ml of the 50 times dilution of sterilized molasses (1% pepton, 0.1% meat extract are added), is called No. 2.

No. 1 and No. 2 are both cultivated for a day at 37°C.

The cultured solution is diluted with a sterilized saline solution in eight steps from 10^{-1} to 10^{-8}. These diluted solutions are cultured at 37°C for two days, on a mannitol salt agar with egg yolk. Afterwards, the number of colonies are counted, and the number of bacteria found in No. 1 and No. 2 are calculated.

RESULTS

The Separation and Identification of *Staphylococcus aureus* and its Cultivation.

The milk is diluted with a sterilized saline solution in seven steps from 10^{-1} to 10^{-7}. These diluted solutions are inoculated to mannitol salt agar with egg yolk and are cultured for approximately 43 hours at 37°C. As a result, seven colonies appeared on the inoculated plate containing the milk that was diluted at 10^{-1}. Using the PS latex and rabbit plasma, these colonies were identified as both being positive. Also, these colonies were yellowish, and the area around them showed the egg yolk reaction. Due to the decomposition of the mannitol and the production of acid, thus the agar culture changed in color from red to yellow. Therefore, it was conclusive that the bacteria present was *Staphylococcus aureus*.

Cultivation of EM and the Growth Control of *Staphylococcus aureus* using EM.

The *Staphylococcus aureus* is cultured in a mannitol salt liquid medium, after which the presence of *Staphylococcus aureus* is carefully counted. Within this, the number of living bacteria counted were 5.8×10^{7} per ml.

When this mannitol salt liquid medium is diluted into 10^{-4} saline solution, the number of bacteria was 5.8×10^{3} per ml.

Sample No. 1 and No. 2 are cultured for one day. In counting the density of the presence of *Staphylococcus aureus*, *Staphylococcus aureus* were not found in No. 1 (with the presence of EM), but were found in No. 2 at 8.2 x 10^7 (Figure 1).

CONCLUSION

This experiment allowed us to confirm that EM successfully prevent growth of *Staphylococcus aureus*, and can sterilize them. However, the conditions in the average barn are different from those of the experiment (temperature, concentration of molasses, and the amount of EM, etc). Therefore eradication of *Staphylococcus aureus* from within a barn is not a given.

It is necessary for more data to be collected, and for further experimentation.

Staphylococcus aureus also grows outside the mammary gland under such conditions as a milking machine in poor condition, and excessive milking, which results in cuts and sores developing on the nipple, as well as milk being spilled on the floor. Infections occur usually during milking and is spread through hands, cloths, and milking units that come in contact with the infected cow. Steps effective in preventing the spread of infective mastitis are disinfecting the nipple, treatment, and destroying the animal.

Figure 1. The growth inhibition of *Staphylococcus aureus* when EM are used

Study #2

Effects of Different EM Solution Addition Ratios and Incubation Methods on Dairy Effluents Treatment

Linxian Liao; Xiaohou Shao; Jun Zeng Xu
College of Agricultural Engineering, Hohai University, Nanjing, China
E-mail: liaolinxian@hhu.edu.cn

Monika Walter
HortResearch, P.O Box 51, Gerald Street, Lincoln, HortResearch, Canterbury, New Zealand

ABSTRACT
In view of heavy pollution caused by dairy effluent, researches on impact of EM activated solution incubation methods, EM addition on dairy effluents treatment are conducted in the labs. Results obtained show that (1) EM addition to dairy effluents can decrease COD and available phosphorus by improving the microorganism activity. (2) Rejuvenated EM solution exhibit biological flocculation characteristics by increasing the amount of sludge sedimentation. (3) Rejuvenated EM solution to dairy effluents anerobically could sufficiently reduce the offensive odour. (4) There is little distinction among effects of different treatments with EM addition ratios and incubation methods on pollutant removal in dairy effluent.

Keywords: Effective Microorganisms®; Dairy Effluent; Addition Ratio; Incubation

INTRODUCTION

In New Zealand, dairy herds typically graze pasture for most of the year. Cows are confined only during milking operations, a total time of 1.5 to 3 hours per day. The controllable manure waste from NZ dairy herds comes from this short period of dairy confinement, and comprises of 6 to 12% of total daily production of manure. Manure from dairy shed confinement is flushed away with water, and this, along with udder washwater, milking plant washwater, and any milk spillage constitutes Dairy Shed Wastewater (DSW). Until the late seventies, most dairy farms in New Zealand discharged DSW untreated into the nearest waterway. Surface water quality in intensive areas was considerably degraded, and Regional Councils for the past 10–20 years have encouraged the installation of two-pond oxidation pond systems to treat DSW prior to discharge. However, the performance of two-pond systems was not good. Therefore, effluent treatment work in New Zealand focused on basic and mechanical system design with a general reliance on microorganisms naturally present.

Effective Microorganisms® (EM), developed by Prof. Teruo Higa, of the College of Agriculture at the University of the Ryukyus in Okinawa, Japan, has been used for processing garbage into fertilizers, treating the effluents leached from garbage, treating the wastewaters discharged from urban areas in lots of countries. EM technology has been proved to be safe, low-cost, effective, and of easy utilization in environmental protection[1-4]. In New Zealand, EM is produced with locally sourced microorganisms by the NZ Nature Farming Society.

The aim of researches is to measure what extent inoculated organisms (EM technology) can benefit New Zealand dairy effluent quality, and to demonstrate the degree to which a microbiological approach might be successful in New Zealand dairy effluent ponds with regard to COD and some nutrient loading, sludge content and bacterial contaminants. The important aspects of EM used in dairy wastewater treatment are about microorganisms' rejuvenation as well as determining the suitable and economical amount of EM[1,3].

MATERIALS AND METHODS

Experiment Design

Lab tests are also designed by use of higher EM activated solution to treat two dairy farms with much higher COD in dairy effluents aerobically with the treatments shown in Table 1. There were two types of EM activated solution: EM1 which was rejuvenated by molasses (EM: molasses: water = 5%: 5%: 90%) and EM2 which was rejuvenated by molasses and dairy effluents (EM2: molasses: dairy effluents: water = 2%: 2%: 5%: 91%). EM activated solution was incubated by molasses and dairy effluent (COD_{Mn} is about 4570mg/L) for 10 days at temperature of 19.5°C. Dairy effluents were collected from ten dairy farms (F1 and F2) in Canterbury (43.55S, 171.22E), New Zealand. Original EM and molasses were supplied by Lincoln University.

Table 1. Design scenarios of dairy effluents treatment by use of EM technology

Treatment	DE[a] (ml)	EM Rejuvenated (ml)	EM:DE
CK	1000	0	0
M 1	1000	1 (EM1)	5/100000
M 2	1000	2 (EM1)	1/10000
M 6	1000	6 (EM1)	3/10000
M D 2.5	1000	2.5 (EM2)	5/100000
M D 5	1000	5 (EM2)	1/10000
M D 15	1000	15 (EM2)	3/10000

[a] DE is the abbreviation of diary effluent.

Monitoring Methods

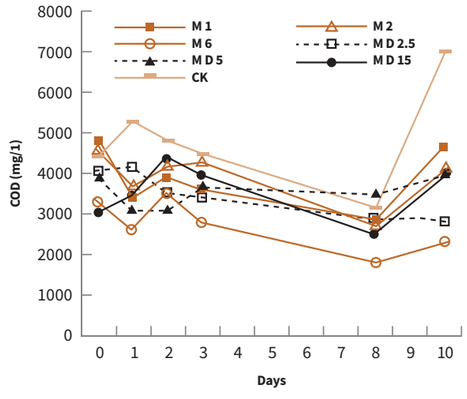
Samples are obtained to measure COD, pH, available P, TSS and VSS almost every day for 7–10 days. COD was determined by manganese reactor digestion method. Conductivity was measured using the probe. Available Phosphorus was determined using Phos Ver 3 with HACH DR/2010 Portable datalogging spectrophotometer. And microorganisms were calculated with plate counting method.

RESULTS AND DISCUSSION

Effects of Different EM Addition Ratios and Incubation Methods on COD Reduction

For the aerobic condition, the reduction of COD is much more significant shown in Figure 1 by 20–50% compared with control after incubation of 6–8 days. The addition of 1/1000 EM treatment in both two dairy farms had higher COD removal than that of 5/10000 EM treatment. After 7 days, the COD removal rates of all treatments have a little decreasing, but the ranges are small. In practical NZ dairy farms, the dairy effluents are usually stored in an anaerobic pond for several days. The results demonstrate addition of EM extended solution to the anaerobic pond could reduce COD of dairy effluents which evidently will decrease the danger of dairy effluent discharge.

Figure 1. The COD reduction of dairy effluents in aerobic condition



Effects of Different EM Addition Ratios and Incubation Methods on Available Phosphorus Reduction

The effects of different EM addition ratios on available phosphorus reduction in dairy effluents is shown in Figure 2. It could be seen at the beginning of the reaction, inorganic P concentration is lower for the EM activated solution addition treatments compared with control. Followed by evident increase for almost

all treatments after 1 day, it was reduced again after 2 days for EM activated solution addition treatments, but it needed 3 days for control to reduce. The results showed that the dairy effluents should keep at least 3 days in the pond systems prior to discharge in order to avoid the pollution problem caused by inorganic P, but if EM activated solution is added, it will be possible to store in just 2 days. The main mechanism for decreasing inorganic P in dairy effluent pond systems is utilized by microorganisms as nutrient as well as adsorption by suspend solid or sludge. The reason of available P increasing might be decomposition of organic P by microorganisms.

Effects of Different EM Addition Ratios and Incubation Methods on Conductivity

Figure 3 shows the effects of different EM addition ratios and incubation methods on conductivity in dairy effluents. It can be seen that conductivity of dairy effluents has a little change as the time goes on for the aerobic incubation test. The reason of conductivity fluctuation in dairy effluents is that the inorganic nutrients are changed.

Effects of Different EM Addition Ratios and Incubation Methods on Suspend Solid

From Table 2, it can be seen at the beginning of reaction the values of TSS and VSS in EM treatments are much lower than those in the control. As time goes on, the TSS and VSS of dairy effluents have a tendency to increase especially for TSS. Moreover, this tendency is always stronger than that of control after adding rejuvenated EM. This shows that some microorganisms in the rejuvenated EM can excrete flocculent material, thereby improving the settlement performance of sludge. Also the activity of flocculation is quite strong at the beginning and the TSS and VSS descends a lot after 10 days.

With the process of time the flocculation of Rejuvenated EM becomes weak, which makes the settlement of sludge worse. But it is still much less than that of the control. Its cause concluded that the flocculent materials are produced to behave biological flocculent after EM is rejuvenated in dairy effluents.

Effects of Different EM Addition Ratios and Incubation Methods on Odour Removal

It can be seen from Table 3 that after 6 days of aerobic incubation, the scale ranges of odor from offensive (++++) to non-offensive, sweet (-) odor. Treatments of adding EM activated solution could remove offensive odor significantly more than control of no EM-1 addition. It could be due to the fact that EM utilizes organic matter which produce odor, and that addition of EM activated solution can change the constituents of microorganisms in dairy effluents which block

Figure 2. Available P change of dairy effluents with EM treatment in aerobic condition

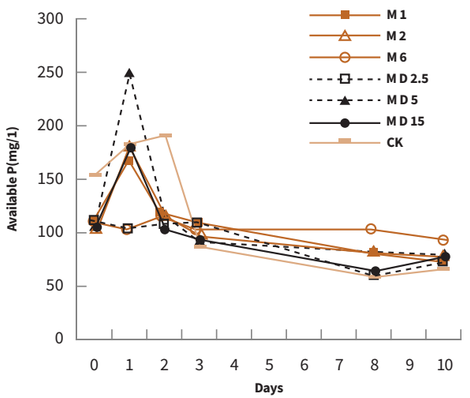


Figure 3. Change of dairy effluents with EM treatment in aerobic condition

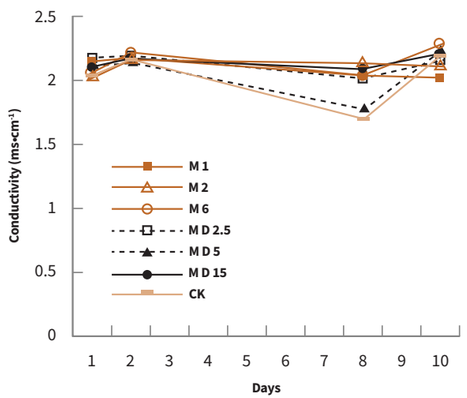


Table 2. Change of TSS, VSS in dairy effluents with EM treatment in aerobic condition

Days	Items	CK	M 1	M 2	M 6	MD 2.5	MD 5	MD 15
0	TSS	6.95	4.50	4.83	2.75	3.04	3.12	2.31
	VSS	3.03	1.87	2.02	1.10	1.26	1.49	0.73
	VSS:TSS	0.44	0.41	0.42	0.40	0.42	0.48	0.32
3	TSS	7.37	8.12	6.56	10.44	10.22	8.89	8.71
	VSS	3.38	3.68	2.95	4.67	4.66	3.99	3.89
	VSS:TSS	0.46	0.45	0.45	0.45	0.46	0.45	0.45
8	TSS	9.86	9.06	9.34	10.73	3.48	8.08	12.59
	VSS	4.59	4.44	7.84	5.04	1.83	2.65	12.59
	VSS:TSS	0.47	0.49	0.84	0.47	0.53	0.33	1.00
10	TSS	7.73	9.97	9.02	10.85	9.92	8.35	8.04
	VSS	3.71	4.66	4.26	5.33	4.65	3.76	3.64
	VSS:TSS	0.48	0.47	0.47	0.49	0.47	0.45	0.45

the growth of offensive bacteria such as Coli and coliform. The results of aerobic incubation also showed that the amount of offensive bacteria was greatly reduced as time processed and treatments of adding EM activated solution were a little bit evident as compared with control.

CONCLUSION

Researches of EM rejuvenation, EM addition to treat dairy effluents are conducted in the lab and field pond systems. Results obtained show that (1) EM addition to dairy effluents can decrease COD and available phosphorus. (2) Rejuvenated EM solution exhibits biological flocculation characteristics through increasing the amount

Figure 4. Change of Microorganisms in dairy effluents with EM treatment in aerobic condition

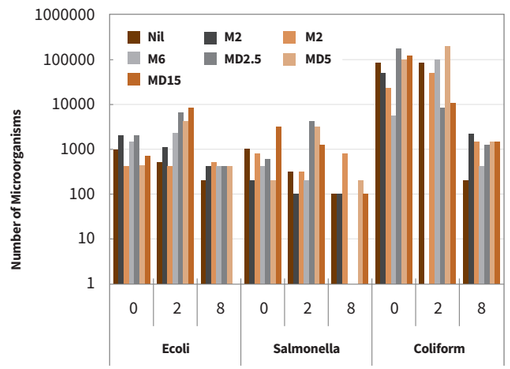


Table 3. Change of TSS, VSS in dairy effluents with EM treatment in aerobic condition

Treatment/ days	0.2	1	2	3	8	10
CK	++++	n.m.	n.m.	n.m.	n.m.	+++
M 1	++++	n.m.	n.m.	n.m.	n.m.	-
M 2	++++	n.m.	n.m.	n.m.	n.m.	-
M 6	++++	n.m.	n.m.	n.m.	n.m.	-
M D 2.5	++++	n.m.	n.m.	n.m.	n.m.	-
M D 5	++++	n.m.	n.m.	n.m.	n.m.	-
M D 15	++++	n.m.	n.m.	n.m.	n.m.	-

n.m. means not measured.

of sludge sedimentation. (3) Rejuvenated EM solution to dairy effluents aerobically could sufficiently reduce the offensive odor. (4) There is little difference among the effects of different treatment, that is, effects of different EM addition ratios and incubation methods on pollutant removal in dairy effluent is not significant in different treatments.

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Study #3

Development and Formulation of Effective Microbial (EM) Technology for Dairy Industrial Effluent Treatment

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ABSTRACT

Dairy Industries produce nearly thousands of litres of effluent waste per day. This waste with high intense foul odour pollutes ecosystem and ground water, harbour pathogens causing health hazards. Various pre-treatments methods are available to neutralise the effects. But, bioremediation with EM technology is ecofriendly and helps to clean up contaminated environments through the use of microorganisms.

EM technology meaning Effective Microorganisms® consisting of beneficial and highly efficient microbes that are non-harmful, non-pathogenic, not-genetically-engineered, or modified (non-GMO), and not-chemically-synthesized. This is proven safe, low-cost, effective, and easily utilized in environmental protection.

In the present study, dairy industry effluent was analysed for microbial content and five different microbes were isolated and labelled as MB1, MB2, MB3, MB4, and MB5. All these were Gram negative, rod shaped and motile. The MB1 colonies displayed Green fluorescence when exposed to UV light; hence it was completely characterized and revealed to be Pseudomonas aureginosa. Biochemical analysis for reducing sugar, protein, lipids was carried out. Also BOD, COD was analysed. Qualitative analysis of Lipids was done by TLC using sprays of ninhydrin solution and molybdic acid which indicated the presence of cholesterol and phospholipids respectively. Quantitative analysis of reducing sugars by Anthrone's test and proteins by Lowry's method gave a result of total carbohydrate content of 500µg/ml and protein content of 100µg/ml.

EM mixture used for effluent treatment included Lactobacillus acidophilus, Chromatium Species, Mucor heimalis, Streptomyces greiceus, Aspergillus Oryzae, Yeast, and Pseudomonas isolated from effluent tank. All the organisms were grown on different media and treated directly on 100 ml of effluent and studied for changes in three parameters viz., pH range 3–4, odour reduction to 1 in the scale of 1–5 (1– least/no odour, 5– foul odour) and clearance of effluent. Except Chromatium sp., others were selected since there was not much change in parameters when Chromatium sp was used. For better results Jaggery solution was used to ferment all the organisms. Sample of effluent used was 400 ml. Odour reduction was observed after 15 days of treatment.

For cost optimization sugarcane juice was used as substrate for fermentation and few more organisms were tried along with the existing EM Mixture. The organisms included lactic acid producing bacteria, Rhodopseudomonas sps and methanotroph. Sugarcane juice was fermented with different combinations of these organisms and treated on 250 ml of effluent sample. Totally 52 different combinations of EM mixture were tried, out of which 4 effective EM combinations (EM1, EM2, EM3 and EM4) were identified based on three parameters. These 4 EM mixtures were studied for scaled up effluent treatment (1 litre) and it was observed that EM1 and EM2 were found to be effective in forming higher clearance in effluent turbidity with reduction in foul odour and pH, there by rendering the effluent fit for safer disposal.

Pot studies were conducted using these EM mixtures for germination and growth of seedlings of Ragi crop. It is observed that the EM treated seeds were first to emerge and establish compared to the untreated control. When added EM solution to the plants, the vegetative growth of the seedling was fast. Also, treated effluent was added to Ragi seedlings. This indicated that EM mixtures stimulate germination and growth related hormones of the crop. These EM1 and EM2 mixtures are maintained as stock cultures in 2.5 litres of sugarcane juice as organic substrate for microbial growth in glass jars. These two EM mixtures can be studied further for field application with proper active formulation for better vegetative growth and yield.

Keywords: EM Technology; Microorganisms; Dairy Industry; Effluent Treatment; TLC; COD; BOD

INTRODUCTION

The Dairy industry involves processing raw milk into products. Dairy industries produce nearly thousands of liters of effluent waste per day. Dairy effluents contain dissolved sugars and proteins, fats, and possible residues as additives. This wastewater has high intense odor (e.g. derivatives of NH_4, H_2S, and CH_3S, etc.) which may cause health hazards. Wastewater originated from dairy operations harbor human pathogens. Hence, pretreatment is of high importance prior to release of this wastewater.

Bioremediation is a new treatment technology to clean up contaminated environments through the use of microorganisms. The natural predatory characteristics of the organisms are utilized to either destroy or change hazardous contaminants to a less harmful form. This technology has proven to recover contaminated sites in a more cost effective manner with less risk to humans than conventional methods.

Effective Microorganisms® (EM) [1] is a mixture of groups of microorganisms that has a reviving action on humans, animals, and the natural environment. And, most of these microorganisms have been used in food processing and in the field of medicine environment. Its safeness has been confirmed from many years of use, and research.

The microbes in EM are non-harmful, non-pathogenic, not-genetically-engineered or modified (non-GMO), and not-chemically-synthesized. EM applications in the field of environment include cleaning polluted waterways, lakes and lagoons, in septic systems, municipal wastewater treatment plants, and landfills/dump sites. The principal micro organisms in EM are Phototrophic Bacteria, Lactic Acid Bacteria, and Yeast. The number of strains utilized, the levels at which they are used in a formula, temperature, and use of other ingredients creates unique formulas.

EM technology is very cost effective and simple in its applications. The integration of EM technology has helped many more farmers adopt sustainable agriculture and an increasing number of farms are converting to organic production system. Thus, there is a real possibility for application of EM in *sustainable management* of agriculture, aquaculture, livestock, human health and hygiene, organic compost from solid waste, flooded forest residues, fish meal and other aquatic weeds, deodorization for fish processing, improved water quality and waste management in the cities.

In the present study an effort has been made to develop and optimise the EM technology for dairy waste treatment and worked further on standardisation of technology for field application.

METHODOLOGY

Isolation of Organisms

Seven different microorganisms were selected [2] and procured from NCIM, Pune. The list is given in the Table 1.

Table 1. Microorganisms procured for the study

Sl. No.	Name	Code	NCIM No.	Maintenance Media
1	*Lactobacillus acidophilus*	LSG	2903	MRS Medium
2	*Chromatium Species*	CSG	2336	MRS Medium
3	*Desulphovibrio desulphuricans*	DLA	2047	Barr's Medium
4	*Streptomyces grieseus*	SLA	2020	MGYP Medium
5	*Mucor hiemalis*	MSK	873	PDA Medium
6	*Yeast*	YLA	-	Nutrient Broth
7	*Aspergillus oryzae*	ASK	-	Sabouraud dextrose broth

An organism from dairy effluent was isolated using skimmed milk agar. Effluent sample was diluted serially and streaked on skimmed milk agar plates. Zone clearance by the organism was observed. After repeated culturing, pure culture thus obtained was observed for various characteristics-morphological characteristic, Grams nature and motility [3].

Biochemical Analysis for Carbohydrates

Qualitative analysis for carbohydrates was based on the Anthrone test, Benedict's test, Barfoed's test, Seliwanoff's test, Bial's test, and Iodine test. Quantitative analysis was carried out using Anthrone test using glucose as standard in the range of 0.2–1.0 mg/ml. Absorbance was recorded at 360nm [4].

Determination of Protein Content

Protein determination was done by Folin-Ciocaltuae method. BSA (0.05–1mg/ml) was used as standard. Different dilutions of the standard and sample were pipette and 2ml of alkaline copper sulphate was added and incubated for 10 minutes. 0.2 ml of reagent Folin Ciocalteau solution (reagent solutions) was added and again incubated for 30 min. Absorbance was recorded at 660 nm [4].

The Determination of Fat in Dairy Effluent

Fat content was analysed by Werner–Schmidt method and percent fat was calculated [5].

Determination of Lipid Content

Lipid content was determined using TLC. Solvents used for separation was Chloroform: Methanol: Water (65:25:4) for polar lipids: phospholipids and galactolipids; Hexane: Di ethyl ether: acetic acid (80:20:1) for neutral lipids. TLC plates were sprayed with Sulphuric acid for total lipids: Iodine vapours, Ferric Chloride spray for cholesterol and cholesteryl esters, Ninhydrin spray for detection of lipids containing amino groups, Anthrone for glycolipids and sulpholipids, Molybdate spray for Phosolipids [6].

BOD for Dairy Effluent

Prepare dilution H_2O by adding 1.0 m each of phosphate buffer solution, $MgSO_4$ solution, $CaCl_2$, and ferric chloride solution to 1 litre of distilled H_2O. Add 2 ml settled sewage and aerate. Determine the exact capacity of 3 BOD bottles. Find out DO of undiluted sample.

Prepare the desired percent mixing by adding sample in dilution H_2O blank. Incubate at fixed temperature for a definite time (20°C–5 days, 30°C–4 days, & 35°C–3 days). Find out DO in both the bottles after incubation and designate mixture as (DO_j), Blank (DO_b) [7].

$BOD = DO_b - DO_j$

COD for Dairy Effluent

Place 50 ml or fraction diluted to 100 ml of sample with distilled water in hard glass bottle and add 25 ml standard potassium dichromate solution. Carefully add 50 ml Conc. H_2SO_4, mix after each addition. Digest the mixture in autoclave for 30 min. Repeat the procedure with 100 ml of distilled water. Transfer the contents to a 500 ml conical flask. Dilute the mixture to about 350 ml. Titrate the excess dichromate with ferrous ammonium sulphate solution using ferrion indicator. The end point is red. Designate the titration value for the sample as B and for distilled water as A [7]. COD = (A–B) C*8*1000/ml sample used. C is normality of ferrous ammonium sulphate.

Table 2. Microorganisms isolated from effluent

Organisms	Code Given	Grams' test	Shape	Motility	Colony
1	MB 1	Gram negative	Rod shape	motile	green
2	MB 2	Gram negative	Rod shape	motile	transparent
3	MB 3	Gram negative	Rod shape	motile	yellow
4	MB 4	Gram negative	Rod shape	motile	shiny
5	MB 5	Gram negative	Rod shape	motile	brownish

RESULTS AND DISCUSSION

ANALYSIS OF DAIRY EFFLUENT

Microbial Analysis

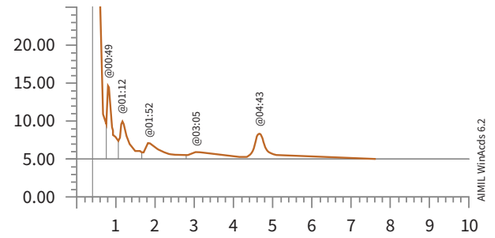
On skimmed milk agar plates five different organisms were observed and were classified based on colony characteristics (Table 2). All the microorganisms observed were grams negative rod shaped and motile with different colony characteristics.

Similar results were observed by K. Rajeshkumar and K. Jayachandra [8] from dairy effluent, where various bacterial strains were identified as *Sporolactobacillus sp*, *Citrobacter* sp, *Pseudomonas sp*, *Alcaligenes sp*, *Bacillus sp*, *Staphalococcus sp*, and *Protease sp*.

Biochemical Analysis

The dairy effluent is analyzed mainly for presence of carbohydrates, proteins, lipids, BOD, and COD. The qualitative analysis for carbohydrates revealed positive for the tests: Benedict's test, Barfoed's test, Seliwanoff's test, Bial's test, Iodine test. Quantitative analysis of carbohydrate by Anthrone method revealed 100 mg/ml of sample. Protein by Lowry's Test revealed 145 μgrams/ml of sample. The lipids extracted by Lipid–Lipid extraction method and determined through Thin Layer Chromatography (TLC) showed presence of Phospholipids, Sulpholipid, and Cholesterol. The Chemical Oxygen Demand was calculated for

Figure 1. Results of GLC



both untreated and treated effluent. There was decrease in BOD and COD level after treatment. Fat percent was observed to be 0.72%.

GLC of extracted lipids from untreated effluent showed presence of palmetic acid, oleic acid, and some other lipids (Figure 1).

The obtained peaks did not match with the standards.

Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) Test of Untreated Effluent Sample

There was almost 50% reduction in COD of sample and 85% reduction in BOD after treatment with EM mixtures. The fermentation of organic compounds in the waste water conducted by EM4 decreased COD gradually which meant that the biochemical reaction in EM4 treated-waste water is increasing due to the higher concentration of oxygen in the wastewater than those in control [13].

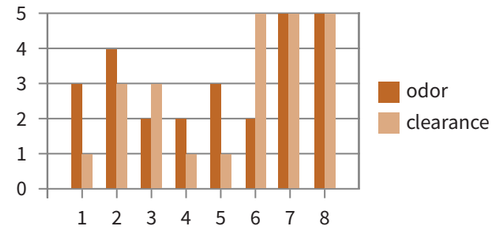
Treatment of Dairy Effluent

One isolated microbe (*Pseudomonas* along with 7 procured microbes were cultured in their specific growth media. The colony counting of *Lactobacillus acidophilus*, Yeast, and *Streptomyces grieseus* was determined (Table 3).

Table 3. Colony count of microbes

Organisms	Dilution	Colony Count/ml
YLA	10^{-5}	32
SLA	10^{-4}	160
LSG	10^{-6}	166

Figure 2. Results of effluent treatment, 1-LSG, 2-CSG, 3-ASK, 4-MSK, 5-YLA, 6-SLA, 7-Mixture, 8-Control



Effect of Individual Organisms on Sample for Odors and Clearance

Effect of each microbe on effluent treatment was determined by inoculating 1 ml of inoculums to 100 ml of effluent water. It was checked for reduction in odor and clearance (Figure 2).

Changes in odor and clearance are graded as 1 to 5 where 1 implies no odor + high clearance, 5 implies high odor + no clearance. The experiment was repeated thrice and similar results were obtained. Since it was observed that CSG has no effect on odor reduction, it was not used in next combination.

A New Combination

A new combination was tried (Table 4). This mixture of microbes was inoculated in 100 ml of Effluent and was kept in shaker for 18 hours at rpm of 120 and at temperature of 30°C.

The result was rated as 2 for odor and 3 for clearance after 18 hrs of inoculation. Effective decrease in odor and clearance was observed, so the same combination was tried for 400 ml of effluent (Table 5).

There was no change in effluent sample after treatment because pH of sample was high (basic). One more parameter was studied i.e. pH.

Table 4. Combination tried

Combination of organisms	LSG	SLA	YLA	ASK	MSK
1	2 ml	2 ml	2 ml	0.2 gm	0.2 gm
2	4 ml	4 ml	4 ml	0.5 gm	0.5 gm
3	6 ml	6 ml	6 ml	1.0 gm	1.0 gm
4	8 ml	8 ml	8 ml	1.5 gm	1.5 gm
5	10 ml	10 ml	10 ml	2 gm	2 gm

According to EM Technology, organisms should grow on some substrate such as jaggery, molasses, sugarcane juice, etc., and then used. Hence different substrates were tried and noted for changes in parameters.

Usage of Jaggery as Substrate

The microbes were maintained in jaggery solution (3%) to form EM stock solution. The Jaggery solution was prepared by adding 116 gm of jaggery in 200 ml of water and pH 7. To this solution, microbes were added and the mixture was allowed to ferment for 2 days.

This fermented solution was inoculated in 400 ml of effluent and for pH, odor, and clearance.

It was seen that there was a change in pH, there was no change in odor and clearance, may be because of use of concentrated Jaggery.

Jaggery solution was diluted with water in the ratio of 1:5 and 1:10 and tried for 100 ml of effluent. There was no change in the parameters tested.

Usage of Sugarcane Juice as Substrate

The microbes were inoculated in sugarcane juice and were fermented for 9 days. Effect of individual microbes were found. This fermented mixture was inoculated in 100 ml of effluent sample. The result showed that MSK has a good effect on odor; YLA has good effect on clearance.

It was observed that few more organisms were required and hence different mixtures were tried. The new combinations were tried (Table 6) and were numbered accordingly. The mixture 1 contains *Rhodobacter sp. Pseudomonas*, *Aspergillus oryzae, Lactobacillus acidophilus Mucor Hiemalis, Streptomyces greiseus*, Yeast, *Chromate sp*, Mixture 1, Mixture 2, and Mixture 3.

From above 10 mixtures, 8 combinations

Table 5. Results of new combination in jaggery solution

Sample + Combination of organism	pH	Clearance	Odor
1	14	5	5
2	14	5	5
3	14	5	5
4	14	5	5
5	14	5	5
Control	14	5	5

Table 6. Results of new combinations in sugarcane juice

Sugarcane juice (75ml) + Consortia (1ml)	pH	Clearance	Odor	Surface Clearance	Sedimentation
7C7	5	5	5	Medium	High
7C7 +8	5	5	5	Medium	High
7C7 +9	4	5	5	Medium	Medium
7C7 +10	5	5	5	Medium	High
7C7 +8+9	3	5	5	Medium	Least
7C7 +9+10	5	5	5	Low	Least
7C7 +8+9+10	4	5	5	Low	Least
7C7 +8+10	4	5	5	Medium	Least
Control	7	5	5	Low	Medium

were tried on 200 ml of effluent and checked for parameters (Table 6).

Now the new combination of 6 microbes along with mixtures 8, 9, 10 were inoculated in 75 ml of sugarcane juice and were fermented for 5 days.

Combination (except CSG) 1. LSG, PLA, MSK, ASK, SLA, YLA, 8, 9, 10 Mixtures.

4 ml of each mixture was inoculated in 250 ml each effluent sample and tested for reduction in odor, clearance, and pH. Out of total 53 combinations, 4 combinations were selected as best based on their ability to reduce odor.

These four EM Mixtures were tired on 1000 ml of effluent. (Table 7).

From above 4 combinations, 6-3+10 and 6-5+9+10 combinations showed good results and are named as EM1 and EM2. These two EM mixtures are maintained in 5 litres of sugarcane juice as stock.

Pot Studies Using Treated Effluent

Ragi seeds were germinated and grown by pouring 5 ml of treated effluent with EM solution and also by pouring normal water twice a week and observed for growth for 15 days. It was observed that EM-1 and EM-2 gave better results (Figure 3).

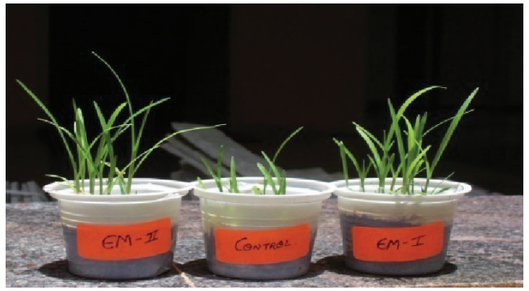
Table 7. Results of new combinations in sugarcane juice

Combination	Odor	pH	Clearance	Sediment
6-5+9+10	3	5	Medium	Low
6+8+9-2-1+10	3	4	Medium	More
6-3+10	2	4	Medium (surface float)	More
6-2+8-1+10	4	5	Medium	Low

CONCLUSION

Industries effluent consisted of five microbes, all of which were gram negative. Biochemical analysis revealed the presence of reducing sugar, protein, and lipids. 500 μg/ml of sugars and 100 μg/ml of proteins was estimated. TLC results indicated presence of cholesterol, phospholipids.

Figure 3. Response of growth of Ragi seedlings in presence of EM Mixture 1, 2, and control (without mixture)



EM technology has been proven to be safe, low-cost, effective, and easily utilized in environmental protection. Organisms used for treatment include *Lactobacillus acidophilus*, *Chromatium Species*, *Mucor heimalis*, *Streptomyces greiceus*, *Aspergillus Oryzae*, Yeast, and *Pseudomonas* isolated from effluent tank. Effect of these organisms were checked for changes in odour, pH, and clearance. *Chromatium sp.* had no effect on changes in parameter and hence it was not selected. Substrates for microorganisms were used. Jaggery solution was used treat effluent sample. There was significant reduction in odour. Sugarcane juice was used as substrate for cost optimization. Odour reduction was observed. With the addition of organisms like lactic acid producing bacteria, *Rhodopseudomonas sps* and methanotroph in the EM mixture. Out of 52 combinations tried, 4 effective EM combinations were identified based on three parameters namely pH range 3–4, odour reduction to 1 in the scale of 1–5 [1– least/no odour, 5– foul odour] and clearance. Out of which two; EM1 and EM2

were found to be effective in forming higher clearance in effluent turbidity with reduction in foul odour and pH, there by rendering the effluent fit for safer disposal. EM treated Ragi crop seeds were studied for germination and growth of seedlings. It is observed that the EM treated seeds were first to emerge and establish as compared to the untreated control and also the vegetative growth of the seedling was fast among EM treated ones. Also the treated effluent was used for growth of seedlings. This indicates EM mixtures stimulate germination and growth related hormones of the crop. These two EM mixtures can be studied in detail and can be recommended for field application with proper active formulation.

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Study #4

Adhesion of Some Probiotic and Dairy *Lactobacillus* Strains to Caco-2 Cell Cultures

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ABSTRACT
The adhesion of 12 different Lactobacillus strains was studied using Caco-2 cell line as an in vitro model for intestinal epithelium. Some of the strains tested have been used as probiotics, and most of them are used in the dairy and food industry. Human and bovine enterotoxigenic Escherichia coli strains were used as positive and negative control, respectively. Bacterial adhesion to Caco-2 cell cultures was quantitated using radiolabelled bacteria. The adherence of bacteria was also observed microscopically after Gram staining. Viability of bacteria prior to adhesion was verified using flow cytometry. Among the tested strains, L. casei (Fyos®) was the most adhesive strain and L. casei var. rhamnosus (Lactophilus®) was the least adhesive strain, approximately 14 and 3% of the added bacteria adhered to Caco-2 cell cultures, respectively. The corresponding values for positive and negative control E. coli strains were 14 and 4%, respectively. The Lactobacillus strains tested could not be divided into distinctly adhesive or non-adhesive strains, since there was a continuation of adhesion rates. The four most adhesive strains were L. casei (Fyos®), L. acidophilus 1 (LC1®), L. rhamnosus LC-705 and Lactobacillus GG (ATCC 53103). No significant differences in the percentage adhesion were observed between these strains. Adhesion of all the strains was dependent on the number of bacteria used, since an approximately constant number of Caco-2 cells was used, indicating that the Caco-2 cell binding sites were not saturated. Viability of bacteria was high since approximately 90% of the bacteria were viable with the exception of L. acidophilus 1 which was 74% viable. Microscopic evaluations agreed with the radiolabelled binding as evidenced by observing more bacteria in Gram-stained preparations of good adhering strains compared to poorly adhering strains. © 1998 Elsevier Science B.V.

Keywords: Probiotics; Adhesion; Lactobacillus; Caco-2 Cell Line

INTRODUCTION

Probiotics are viable microbes in food and feed supplements which beneficially influence the microbial balance of the host (Fuller, 1989). Some probiotics can be isolated from faeces from days up to a few weeks after oral consumption of the microorganism. Probiotics are believed to temporarily colonise the intestine by adhering to intestinal surfaces. Therefore, the adhesive ability of bacteria to intestinal cells has been considered as one of the selection criteria for probiotic strains (Brassart et al., 1994; Salminen et al., 1996b).

Because it is difficult to investigate bacterial adherence in vivo, adhesion has been studied using intestinal cell lines of human origin in culture as in vitro models for intestinal epithelium. One of these intestinal cell lines is the Caco-2 cell line which was originally isolated from a human colon adenocarcinoma (Fogh et al., 1977). The Caco-2 cell line spontaneously differentiates under standard culture conditions and the differentiated cells express characteristics of mature enterocytes (Pinto et al., 1983). In this study, Caco-2 cell cultures were used to investigate the adhesion of 12 different *Lactobacillus* strains. Some of the strains tested were known to adhere to Caco-2 cells, for example *L. acidophilus* LA1 (Bernet et al., 1994; Brassart et al., 1994), *Lactobacillus* GG (Elo et al., 1991; Coconnier et al., 1992; Lehto and Salminen, 1997) and *L. rhamnosus* LC-705 (Lehto and Salminen, 1997). Human enterotoxigenic *Escherichia coli* H10407 (Deneke et al., 1984) and bovine enterotoxigenic *E. coli* B44 strains (Deneke et al., 1983) were used as positive adhesive and as negative adhesive control strains, respectively (Elo et al., 1991).

Most of the *Lactobacillus* strains tested are used in the dairy industry and some of them are used as probiotic strains. Six out of 12 strains were isolated from commercial products and are cited in this study, as they are referred to in the products which contain them. Among the *Lactobacilli* tested, beneficial effects for human health have been reported for *Lactobacillus* strains, *L. acidophilus* LA1, *L. casei* Shirota, and *Lactobacillus* GG (Lee and Salminen, 1995; Salminen et al., 1996a,b).

The aim of this study was to determine the differences in adhesion of probiotic and dairy *Lactobacillus* strains to Caco-2 cells in culture in vitro and to observe whether strains with reported health effects are more adhesive than the other strains tested.

MATERIALS AND METHODS

Bacterial Strains, Growth Conditions, and Radiolabelling

Lactobacillus strains identified by API 50 CHL (4.0) (Bio Mérieux, l'Etole, France) (Table 1) and *E. coli* strains were kindly provided by Dr. M. Saxelin (Valio Ltd., Helsinki, Finland). API 50 CHL system identifies bacterial strains according to their ability to ferment carbohydrates. All *Lactobacillus* strains were grown (a 0.2% inoculum from stocks stored at -70°C in 40% glycerol) in de Man, Rogosa and Sharpe (MRS; Merck, Darmstadt, Germany) broth at 37°C for 18–20 h. For metabolic radiolabelling, 5 μl ml^{-1} of methyl-1,2-[^{3}H]-thymidine (121 Ci mmol^{-1}; Amersham International, Buckinghamshire, UK) was added to the broth. To remove the excess radiolabel after growth, bacteria were centrifuged (1000 x *g*) and the pellet was washed twice with phosphate-buffered saline (PBS; 10 mmol l^{-1} phosphate, pH 7.2). The optical density at 600 nm of a 1:5 dilution of bacterial suspensions were adjusted to 0.5 ± 0.05 to give approximately between 3 x 10^8 and 1–2 x 10^9 colony-forming units (CFU) ml^{-1}. The colony-forming units were determined by plating serial 10-fold dilutions of corresponding non-labelled bacterial suspensions with MRS agar. To study the concentration dependence of *Lactobacillus* adhesion, the bacterial suspensions were diluted in PBS.

Human enterotoxigenic *E. coli* (H10407) and bovine enterotoxigenic *E. coli* (B44) were grown at 37°C for 18–20 h in Luria-Bertani broth (Sigma Chemical Co., St. Louis, MO, USA) containing radiolabel. After growth, the bacteria were washed with PBS containing 0.1% (w/v) Na-azide (to kill the bacteria) and resuspended in PBS. This treatment prevented the *E. coli* from invading the cell, however it did not prevent binding to the cell surface (Finlay and Falkow, 1989). Bacterial suspensions of *E. coli* strains H10407 and B44 contained approximately 4 x 10^8 CFU ml^{-1} (A_{600} 1.0 ± 0.1) and 3 x 10^8 CFU ml^{-1} (A_{600} 0.8 ± 0.1), respectively.

Caco-2 Cell Culture

The Caco-2 cell line (ATCC HTB 37) was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were cultured in Dulbecco's modified Eagle's minimal essential medium (DMEM; HyClone Laboratories Inc., Logan, UT, USA) supplemented with 10% (v/v) heat-inactivated (30 min, 56°C) fetal calf serum (HyClone), 2 mM L-glutamine (Sigma), 100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin (Biological Industries, Kibbutz Beth Haemek, Israel) at 37°C in an atmosphere of 10% CO_2/90% air. For adhesion assays Caco-2 monolayers were prepared on glass coverslips placed in 24-well tissue culture plates.

Cells were seeded at a concentration of 5 x 10^5 cells per well to obtain confluence and maintained for 2 weeks prior to use in adhesion assays. The cell culture medium was changed every other day and replaced by fresh non-supplemented DMEM at least 1 h before the adhesion assay.

In Vitro Adhesion Assay

The adherence of bacterial strains to Caco-2 cell cultures was examined by adding 50 µl of a radiolabelled bacterial suspension to three wells containing the Caco-2 monolayer (1 ml of DMEM in the well). After incubation at 37°C for 1.5 h the Caco-2 cell cultures were washed three times with 1 ml of PBS and treated with 100 µl of 0.9 M NaOH at 37°C overnight to lyse both the Caco-2 cells and bacteria. The lysed cells were then mixed with scintillation liquid and the radioactivity was measured by liquid scintillation. The adhesion ratio (%) was calculated by comparing the radioactivity of the original bacterial suspension that was added (triplicate 50-µl samples) to the final counts from the lysed cells. All 12 strains were tested at the same time in triplicate in three independent experiments, and in triplicate twice in groups of four strains per experiment. A total of five triplicate measurements were made for each strain.

In the case of studies with non-labelled bacteria, the bacterial suspensions were prepared and used in adhesion assays exactly as the suspensions containing radiolabelled bacteria. After adhesion the Caco-2 monolayers were washed three times and fixed for 30 min at room temperature with 3% (w/v) paraformaldehyde in PBS. After fixing, the Caco-2 monolayers were washed three times with PBS, dried in air and Gram-stained. The Gram-stained preparations were prepared in triplicate once or twice in the case of some strains. Adherent bacteria were detected microscopically by counting 15 randomised fields per coverslip.

Number and Viability of *Lactobacillus* Cells Measured by Flow Cytometry

The number of *Lactobacillus* cells was measured by flow cytometry using non-labelled bacterial suspensions prepared in a similar manner as radiolabelled bacterial suspensions. The flow cytometer (Coulter Electronics EPICS XL) was equipped with an air-cooled 488-nm argon-ion laser at 15 mW. For viability testing, bacteria were stained using LIVE/DEAD® Bac Light™ Bacterial Viability Kit (Molecular Probes Inc., Eugene, OR, USA) and viability of bacteria was measured in a flow cytometer. All strains were tested in duplicate three times (n = 3).

Statistical Analysis

Statistica program version 5.1 (Statsoft Inc., Tulsa, OK, USA) was used to analyse the data. Tukey's test after the analysis of variance (ANOVA) or Student's t-test was used to identify differences among tested strains.

RESULTS

L. casei (Fyos®) was the most adhesive strain in this study since approximately 14% of the added bacteria were bound to Caco-2 cell cultures (Table 1). However, the adhesion of *L. casei* (Fyos®) did not significantly differ from the adhesion of *L. acidophilus* 1, *L. rhamnosus* LC-705 and *Lactobacillus* GG. When compared to the positive *E. coli* H10407 control, these were the only strains which did not significantly differ from the positive control. The adhesion of *L. casei* (Fyos®), *L. acidophilus* 1, *L. rhamnosus* LC-705, *Lactobacillus* GG, *L. rhamnosus* ATCC 7469, *L. rhamnosus* (human faecal isolate) and *L. plantarum* ATCC 8014 was significantly better than the adhesion of the negative *E. coli* B44 control ($P < 0.05$).

The adhesion of all *Lactobacillus* strains tested was dependent on the amount of bacteria in each well (Fig. 1). The number of bacteria bound to Caco-2 cell cultures was directly related to the number of bacteria added. A saturation level was not reached with the bacterial concentrations used in this study.

When Gram-stained preparations were observed visually, those strains identified as *L. rhamnosus* strains by API system (Table 1) adhered in chains and bacterial clusters were detected (Fig. 2). *L. acidophilus* 1 appeared to adhere individually (Fig. 2) and strains identified as *L. paracasei* (Table 1) adhered in pairs or in short chains.

The counting of adherent bacteria for strains that had high adherence could not be done reliably because bacteria formed clusters (Fig. 2). For example, when adherent *Lactobacillus* GG were counted the number of bacteria per field varied from 30 to approximately greater than 500. On average, approximately 200 bacteria per field were observed (n = 2). Counting of poorly adhering strains showed that less bacteria adhered per field compared to good adhering strains. For example, in the case of *L. casei* Shirota 18 (S.D. = 2, n = 2) bacteria adhered per field.

Lactobacillus cells survived well during the washes after growth, since the viability of most of the strains varied between 89 and 98% (data not shown). Only the viability of *L. acidophilus* 1 was significantly reduced ($P < 0.01$) compared to the viability of other strains, since only 74% of the bacteria were viable (data not shown).

The number of bacteria obtained by

Table 1. API identifications for *Lactobacillus* strains and adhesion of radiolabelled *Lactobacillus* and *E. coli* strains to human intestinal epithelial Caco-2 cell cultures

Bacterial Strain	API CHL (4.0) Identification	Adhesion (%)[a] , mean ± S.D. (n = 5)
L. casei (Fyos®, Nutricia)	*L. rhamnosus*	14.4 ± 3.9*
L. acidophilus 1 (LC1®, Nestlé)	*L. acidophilus*	12.6 ± 2.5*
L. rhamnosus LC-705	*L. rhamnosus*	9.8 ± 1.8*
Lactobacillus GG ATCC 53103	*L. rhamnosus*	9.7 ± 3.3*
L. rhamnosus ATCC 7469	*L. rhamnosus*	7.4 ± 1.5*
L. rhamnosus (human faecal isolate)	*L. rhamnosus*	7.2 ± 1.6*
L. plantarum ATCC 8014	*L. plantarum*	6.7 ± 1.4*
L. casei (BIO®, Danone)	*L. paracasei*	4.8 ± 1.3**
L. casei Imunitass (Actimel®, Danone)	*L. paracasei*	4.4 ± 1.1**
L. casei 01 (Starter culture, Chr. Hansen)	*L. paracasei*	4.3 ± 1.3**
L. casei Shirota (Yakult®, Yakul)	*L. paracasei*	3.2 ± 0.52
L. casei var. *rhamnosus* (Lactophilus®, Laboratoires Lyocentre)	*L. rhamnosus*	2.6 ± 0.69
E. coli B44 (negative control)		4.0 ± 0.43
E. coli H10407 (positive control)		14.2 ± 5.0

S.D., standard deviation; n = number of independent experiments.
[a]Adhesion ratio (%) was calculated by comparing the radioactivity of the bacteria added to the radioactivity of the bacteria bound to the Caco-2 cell cultures. The number of the bacteria added varied between 2 x 10^8 and 1 x 10^9 CFU ml^{-1} (measured by flow cytometry). *Not significantly different from the positive control (Student's t-test, $P > 0.05$). **Not significantly different from the negative control (Student's t-test, $P > 0.05$).

Figure 1. Concentration dependence of adhesion of 12 *Lactobacillus* strains to Caco-2 cell cultures. The total number of the bacteria added (at the highest concentration) was measured using flow cytometry. The number of bacteria bound was calculated by comparing the radioactivity of the bacteria bound to the radioactivity of the bacteria added. Each data point is a mean for the combined data obtained with triplicate samples in two separate experiments.

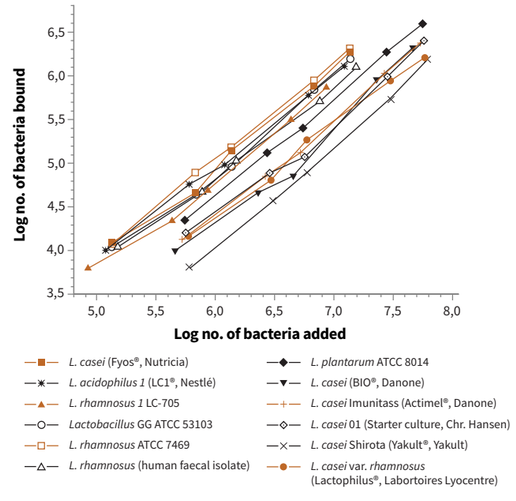
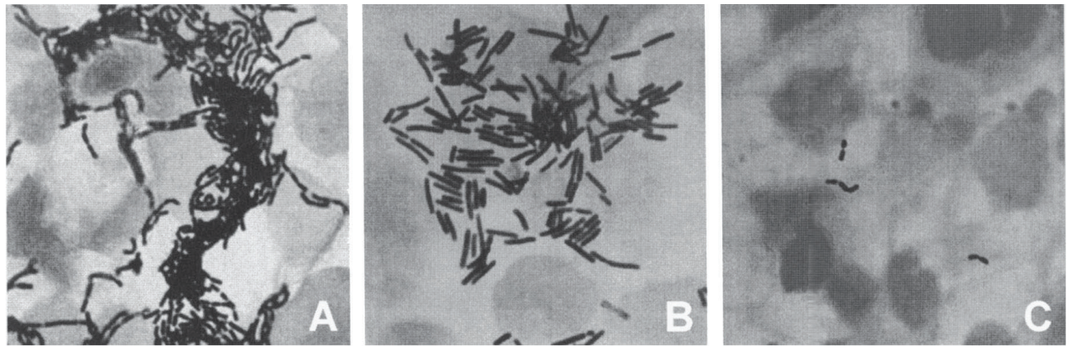


Figure 2. Adhesion of some non-labelled *Lactobacillus* strains to Caco-2 cell cultures observed using light microscopy after Gram-staining. (A) *L. casei* (Fyos®, Nutricia). (B) *L. acidophilus* 1 (LC1®, Nestlé). (C) *L. casei* var. *rhamnosus* (Lactophilus®, Laboratoires Lyocentre)



flow cytometry and by plate counting were significantly correlated ($r = 0.79$, $P < 0.01$). The adhesion and viability of the *Lactobacillus* strains were not correlated.

DISCUSSION

Because bacterial adhesion to epithelial cells has been considered as one of the selection criteria for probiotic strains, the Caco-2 cell line has been used as an in vitro model for intestinal epithelium and the cell line has been used to screen for adhesive strains (Elo et al., 1991; Chauvière et al., 1992; Bernet et al., 1993, 1994). In this study, the adhesion of several probiotic and dairy *Lactobacillus* strains to Caco-2 cell cultures was investigated using a quantitative assay based on radiolabelled bacteria. Microscopic examination of adhesion between Caco-2 cell cultures and non-labelled bacteria was performed to support the data obtained from radioactive assays. The subjective microscopic observations agreed with the radioactivity measurements, since more bacteria were observed to adhere in Gram-stained preparations of good adhesive strains than in poorly adhering strains (Fig. 2.). Judged by radioactivity measurements, *L. casei* (Fyos®), *L. acidophilus* 1, *L. rhamnosus* LC-705 and *Lactobacillus* GG were the most adhesive strains (Table 1). Using the measured adhesion level of the negative control *E. coli* B44 as an indicator of non-specific binding, the adhesion of *L. casei* (BIO®), *L. casei* Imunitass, *L. casei* 01, *L. casei* Shirota and *L. casei* var. *rhamnosus* could be considered non-specific binding. The mechanisms of adhesion were not studied. Thus, it was not possible to define which strains bound due to specific interactions mediated by adhesin(s) and which strains bound by non-specific interactions (for reviews, see Beachey, 1981; Busscher and Weerkamp, 1987).

Surprisingly, it was observed, that the bacterial suspensions adjusted to an absorbance 0.5 contained less bacteria in the case of the most adhesive strains (Fig. 1). This result showed that the O.D. per cell was higher with these strains suggesting that the cells may have surface components which affect to the turbidity of bacterial suspension.

Chauvière et al. (1992) studied the adhesion of *L. rhamnosus* strain ATCC 7469 and *Lactobacillus* GG among many other *Lactobacillus* strains to the Caco-2 cell line. *Lactobacillus* GG was found to adhere at a moderate level (125 adhered bacteria per 100 Caco-2 cells) and *L. rhamnosus* ATCC 7469 adhered poorly (six adhered bacteria per 100 Caco-2 cells) when compared to the highly adhesive *L. acidophilus* LB (210 adhered bacteria per 100 Caco-2 cells). In their study the different lactobacilli were allowed to adhere in the presence of the spent culture broth, and thus the results are not strictly comparable to the results of this study. In this study, *L. rhamnosus* ATCC 7469 was ranked fifth among the 12 strains studied (Table 1).

Adhesion of all *Lactobacillus* strains was concentration dependent (Fig. 1), indicating that the same fraction of added bacteria adhered at different bacterial concentrations. This result is in agreement with the findings of Greene and Klaenhammer (1994), who reported similar concentration dependence with some *Lactobacillus* strains. In this study, the highest bacterial concentrations used did not saturate the binding capacity of the Caco-2 cells. This finding can be due to the bacterial clusters formed at high concentrations (Fig. 2). The linear adhesion at low bacterial concentrations was not further investigated. Therefore, it is not possible to suggest whether it is due to the differences between individual bacteria or the Caco-2 cells. It is important to consider the observed concentration dependence of adherence when adhesion of different strains is compared using direct microscopic counting. Since the amount of bound bacteria is dependent on the number of bacteria added, the amount of bacteria added should be kept constant in order to compare the binding of different strains.

The differences in adhesion between strains were not related to differences in viability among *Lactobacillus* strains, since only *L. acidophilus* 1 had a significantly reduced viability compared to other strains. The observed adhesion of *L. acidophilus* 1 was not corrected with the reduced viability, because the binding capacity of dead bacteria was not known. Flow cytometry appeared to be useful in measuring the viability and the number of bacteria in cell suspensions, because it could be performed rapidly when compared to methods based on bacterial growth, like plate counting.

In the present study, the tested *Lactobacillus* strains were not separated into groups of adhering and non-adhering strains. Instead, a continuous range of adhesion capacity was measured between the most adherent *L. casei* (Fyos®) and the least adherent *L. casei* var. *rhamnosus* strain. Generally, the strains which were identified as *L. rhamnosus* strains by API identification system appeared to be more adhesive when compared to those strains which were identified as *L. paracasei* strains (Table 1). Commercial probiotic strains with reported health effects did not adhere more effectively than other strains, although *L. acidophilus* 1 and *Lactobacillus* GG were among the best adhering strains. The probiotic *L. casei* Shirota adhered poorly in this study, as did the probiotic *L. acidophilus* NCFB 1748 in a study reported by Elo et al. (1991). Sarem-Damerdji et al. (1995) reported that both *L. acidophilus* NCFB 1748 and *Lactobacillus* GG exhibited an intermediate colonisation to human colonic tissue samples in vitro. Therefore, the adhesion to Caco-2 cell cultures should not be used alone to estimate which strains are adhesive or have beneficial health effects in vivo.

Although the conclusions drawn from results of in vitro studies cannot be directly related to in vivo situation, there is evidence relating adhesion to the temporary colonisation of human intestinal tract. Crociani et al. (1995) demonstrated that the ability of some bifidobacteria strains to adhere to Caco-2 cell cultures and colonise human volunteers was similar. The adhesion of *Lactobacillus* strains in this study can be directly compared to each other by assuming, for example, that the number of bacteria added to the Caco-2 cell culture is exactly 1×10^8. Based on 1×10^8 bacteria added to the cell culture, the number of bacteria adhering varied between 1.4×10^7 and 2.6×10^6 for the most adhesive strain *L. casei* (Fyos®) and least adhesive strain *L. casei* var. *rhamnosus*, respectively. If this result is directly comparable with the in vivo situation, five times more *L. casei* var. *rhamnosus* than *L. casei* (Fyos®) have to be consumed to have the same number of organisms adhere to the intestinal epithelium. However, the survival of each strain in vivo in the gastric juice has to be taken into account. Therefore, preliminary studies with Caco-2 cell line can be useful to collect the background information of probiotic strains in order to perform dose–response studies with human volunteers (Saxelin et al., 1991, 1993; Saxelin, 1997).

CONCLUSIONS

Different adhesion capacities of *Lactobacillus* strains to Caco-2 cell cultures were observed, but the strains could not be strictly divided into good adhering and poorly adhering strains based on their present use as either probiotic strains with reported health effects or as dairy strains.

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Batch Number:

Ca300™ is a naturally fermented live microbial product. **Ca300™** is safe for humans, animals, and the whole planet.

Store **Ca300™** out of direct sunlight.

Store in a container at room temperature. Do not freeze.

Best Used By:

White flakes may appear on the surface and are natural due to live cultures. Some sediment at bottom of container is natural.

TeraGanix

Made in the USA
Distributed exclusively by TeraGanix, Inc.
Alto, Texas 75925
www.teraganix.com

TeraGanix Pro™

Ca300™

Direct Fed Microbial for Cattle

■ 1 Gallon (4L) ■ 5 Gallon (19L)
■ 55 Gallon (208L) ■ 275 Gallon (1041L)

EM CERTIFIED PRODUCT

Original | Authentic
Effective Microorganisms®

Uses

A direct fed microbial for use in cattle drinking water.

Directions

Add to drinking water of cattle continuously at a 1:2000 ratio. (In a 1:128 medicator, this will equal 1 quart **Ca300™** to 5 gallons of water).

Active Ingredients

Contains live naturally occuring microbes, Lactobacillus casei, Guarantee of 1 million CFU/ml

Inactive Ingredients

99% Water

0	Health
0	Flammability
0	Physical Hazard
0	Personal Protection

Batch Number:

Fb300™ is our liquid probiotic solution for cattle foot baths. Apply **Fb300™** in place of other toxic chemicals in the foot bath water.

Keep this and all cleaning products out of reach of children. Test on delicate surfaces for dilution rates.

Contains no preservatives, phosphorus, detergents, or surfactants.

Best used within one year after opening. Store at room temperature in a sealed container out of direct sunlight.

Best Used By:

TeraGanix

Made in the USA
Distributed exclusively by TeraGanix, Inc.
Alto, Texas 75925
www.teraganix.com

TeraGanix Pro™

Fb300™

Foot Bath Treatment for Cattle

■ 1 Gallon (4L) ■ 5 Gallon (19L)
■ 55 Gallon (208L) ■ 275 Gallon (1041L)

EM CERTIFIED PRODUCT

Original | Authentic
Effective Microorganisms®

Uses

Alternative foot bath treatment to synthetic chemicals.

Directions

Intended to treat 200 cows after which it is recommended that the foot bath solution be discarded and replaced with a fresh **Fb300™** foot bath solution. Animals may be walked through a foot bath of 1% (add 1/2 gallon **Fb300™** to 50 gals water) to 5% (add 2.5 gals **Fb300™** to 50 gals water) aqueous solution with an immersion time of 5 to 20 min twice daily for a period of time as prescribed by a veterinarian. Keep foot baths clean during treatment period. Do not allow cattle to drink from foot baths. Follow instructions under Storage and Disposal when solutions are discarded at end of treatment period.

If gallon capacity of foot bath is unknown, use the **following formula** to calculate:

Inside Length (in.) X Inside Width (in.) X Fluid Height (in.) / 231 = gallon capacity of foot bath

Ingredients

Purified Water, Certified Organic Vinegar, Yucca Extract*, Certified Organic Grain Alcohol, Organic Sugars, EM•1® Waste Treatment*, and d-Limonene. *OMRI Listed Ingredients.

Disposal

Drain disposal.
Recycle packaging.

0	Health
0	Flammability
0	Physical Hazard
0	Personal Protection

Uses

Floors, walls, kitchen appliances, hard surfaces, and carpet. Also safe to use in livestock trucks, pits, and parlors.

Directions

Dilute **Apc500™** with clean water. Fill a trigger spray bottle and spray surface until visibly wet. Soak area if desired. Wait 1-10 minutes and wipe dry with squeegee or microfiber cloth. Repeat as necessary.

Suggested Dilution Rates:

JOB	DILUTION RATE **Apc500™** : Water	MACHINES **Apc500™** : Water
Stone, Tile, Marble, & Concrete	2-6oz per gallon 1:20-64	1-2oz per gallon 1:64-128
Carpets	2-4oz per gallon 1:32-64	2-6oz per gallon 1:20-64
Stoves & Hoods	4-6oz per gallon 1:20-32	4-6oz per gallon 1:20-32
Wood	1-2oz per gallon 1:64-128	1-2oz per gallon 1:64-128
Glass & Plastics	1/2oz-1oz per gallon 1:32-1:64	1/2oz-1oz per gallon 1:32-1:64

Ingredients

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Disposal

Drain disposal.
Recycle packaging.

0	Health
0	Flammability
0	Physical Hazard
0	Personal Protection

Apc500™ is a safe, all-natural low-foaming all-purpose cleaner, degreaser, and deodorizer. Regular use of **Apc500™** will not corrode equipment. **Apc500™** is safe on all surfaces and may be used in steam or mechanical cleaning machines. For best results, use microfiber cloths when cleaning.

Keep this and all cleaning products out of reach of children. Test on delicate surfaces for dilution rates.

Contains no preservatives, phosphorus, detergents, or surfactants.

Best used within one year after opening. Store at room temperature in a sealed container out of direct sunlight.

BATCH NUMBER:

BEST USED BY:

TeraGanix

Made in the USA
Manufactured by TeraGanix, Inc.
Alto, Texas 75925
www.teraganix.com

Batch Number:

Best Used By:

Wt1000™

Waste Treatment Concentrate

Original Authentic

Effective Microorganisms®

☐ 5 Gallon (19L) ☐ 55 Gallon (208L) ☐ 275 Gallon (1041L)

USES: For Environmental Applications.
RV, boat, bus, Portable Toilets Weekly 1:128 (1oz / Gal)
Septic Systems Monthly 1:1000 (0.75 tsp / Gal)
Ponds & Water Features Monthly 1:10,000
Composting Each Preparation 1.5 Gal Per Cubic Yard
Livestock odors 2-3 times Per Week 1:64 (2oz / Gal)
Waste Lagoons Monthly 1:10,000

Do not store Wt1000™ in direct sunlight.
Store in closed container at room temperature.
Do not freeze.

White Flakes may appear on the surface due to live cultures. Sediment at bottom of bottle is natural.

Active Ingredients: Microorganisms ... 1 million colony forming units/cc (units/ml) 1%: Lactobacillus casei, Rhodopseudomonas palustris, Saccharomyces cerevisiae.

Inactive Ingredients: 96% Water and 3% Molasses.

0	Health
0	Flammability
0	Physical Hazard
0	Personal Protection

TeraGanix

Made in the USA
TeraGanix, Inc.
19371 US Highway 69 So
Alto, TX 75925
866.369.3678
www.TeraGanix.com

Batch Number:

Best Used By:

Ag1000®

Organic

Agricultural Concentrate

Original
Authentic

Effective Microorganisms®

☐ 5 Gallon (19L) ☐ 55 Gallon (208L) ☐ 275 Gallon (1041L) ☐ Bulk

USES:
For all soils and plants as part of a crop nutrient program.

APPLICATIONS GUIDELINES:
Apply to soils using irrigation, pre-plant incorporated, or pre-emerge, at a rate of 10 to 45 gallons per acre.

Apply post harvest to crop residues at a rate of 5-15 gallons per acre.

Do not store Ag1000® in direct sunlight. Store in closed container between 40ºF and 100ºF. Do not freeze.

White flakes may appear on the surface due to live cultures. Sediment at bottom of container is natural.

CONTAINS NON PLANT FOOD INGREDIENTS
Active Ingredients:
Microorganisms ... 1 million colony forming units/cc (units/ml) 1%: Lactobacillus casei.
Inactive Ingredients: 99% Water
Information regarding the contents and levels of metals in this product is available on the internet at http://www.aapfco.org/metals.htm

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Alto, TX 75925
866.369.3678
www.TeraGanix.com

0	Health
0	Flammability
0	Physical Hazard
0	Personal Protection

Batch Number:

Best Used By:

Ag1000®

Agricultural Concentrate

Original
Authentic

Effective Microorganisms®

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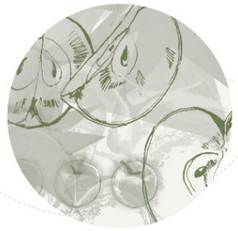
866.369.3678

0	Health
0	Flammability
0	Physical Hazard
0	Personal Protection



OMRI Listed®

The following product is OMRI Listed. It may be used in certified organic production or food processing and handling according to the USDA National Organic Program Rule.

Product
Ag1000 Organic Agricultural Concentrate

Company
TeraGanix, Inc.
Mr. Eric Lancaster
14193 US HWY 69 S.
Alto, TX 75925

Status
Allowed

Category
NOP: Compost Inoculants
NOP: Microbial Inoculants

Issue date
27-Sep-2013

Product number
ema-4342

Class
Crop Management Tools and Production Aids

Expiration date
01-Dec-2016

Restrictions
Not applicable.

Peggy Miars
Executive Director

Product review is conducted according to the policies in the current *OMRI Policy Manual*® and based on the standards in the current *OMRI Standards Manual*®. To verify the current status of this or any OMRI Listed product, view the most current version of the *OMRI Products List*® at OMRI.org. OMRI listing is not equivalent to organic certification and is not a product endorsement. It cannot be construed as such. Final decisions on the acceptability of a product for use in a certified organic system are the responsibility of a USDA accredited certification agent. It is the operator's responsibility to properly use the product, including following any restrictions.

OMRI LISTED
For Organic Use

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