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Selective Antimicrobial Activity of ASAP-AGX-32 Silver Solution Against Probiotics

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1. Introduction and Purpose

Since our research began years ago on the antibacterial activity of ASAP silver solutions, we have observed the bactericidal action against all microorganisms exposed to it—almost all of them being pathogens of varying biological levels. Due to our consistent observation of the ASAP silver solutions being bactericidal to varying degrees against all bacteria we have tested it with, we were skeptical about the hypothesis that silver solution could be selectively bactericidal against pathogens, but have little or no effect against probiotics.

Probiotics are non-pathogenic, non-toxigenic microorganisms that are able to survive the transit through the stomach and colonize the stomach and gastrointestinal tract to inhibit the colonization of any potentially pathogenic bacteria ingested. The two most recognized and used probiotics are *Lactobacillus* and *Bifidobacterium* species—found in yogurts and other dairy products. Probiotics are used frequently as prophylactic treatment in conjunction with the use of antibiotics in order to prevent the diarrhea that is so commonly caused by the use of antibiotics and are also frequently taken as a dietary supplement for the prevention of gastrointestinal bacterial infection.

Due to the lack of sufficient broad-spectrum antibiotics, and the negative side effects associated with many antibiotics, science has continued its search for an antibiotic that has no notable side effects but is an efficient bacterial killer. The ASAP Silver solution appears to be unique in its ability to kill a large number of microorganisms, including plague, tuberculosis, and anthrax, while at the same time apparently having very little antimicrobial effect against the organisms most commonly used as probiotics.

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After years of research pertaining to its antimicrobial activity, scientists have been testing other ways to harness the power of silver in preventative medicine. So the question arises regarding the antimicrobial activity of ASAP silver solution against probiotics. Scientists at Viridis Biopharma have conducted limited research which shows that ASAP silver solution is ineffective against probiotic mixtures. However, their research did not use the standard MIC/MBC test methods, they did not indicate the results using pathogenic controls, nor was each microorganism tested individually with ASAP silver solution, so we felt the need to bolster the research with the above procedures in order to more effectively examine the claim that the ASAP AGX 32 Silver Solution was not antimicrobial against the probiotics.

This report demonstrates that ASAP-AGX-32 silver solution has very limited antimicrobial activity against some of the individual strains found in the probiotics, but is ineffective against others when tested extensively with American Biotech Laboratory's 32ppm silver using the standard protocols listed above.

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2. Aim

To demonstrate the selective antibacterial activity of ASAP-AGX-32 silver solution against probiotics previously tested by Viridis Biopharma. Each microbe will be tested using the broth macrodilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) will be tested in plastic test tubes.

The bacteria tested in this research are:

ATCC 4356 *Lactobacillus acidophilus*

ATCC 12315 *Lactobacillus delbrueckii subsp. lactis*

ATCC 29521 *Bifidobacterium bifidum*

ATCC 15707 *Bifidobacterium longum*

ATCC 19258 *Streptococcus thermophilus*

Streptococcus lactis

ASAP-AgX-32ppm Solution

Manufactured by American Biotech Labs in Alpine, UT

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3. Experiment Design

All but one of the isolates were purchased directly from ATCC and cultured in the media recommended for each specific strain by ATCC on their product information sheet.

Each of the six isolates had the broth macrodilution method of MIC/MBC performed on them in their respective media in 17mm x 12mm polystyrene capped tubes. Polystyrene were used instead of glass because silver tends to interact with glass, thereby lowering the concentration of silver available for antimicrobial activity.

ASAP-AGX-32 silver solution containing 32ppm of silver were used and serially diluted in the media specific to the organism, then each tube were inoculated with overnight culture of one organism diluted to 0.5 of the McFarland standard with sterile media. Thereafter, the tubes were incubated for 24 – 48 hours and observed for growth. Pictures were taken of the tubes after incubation to exhibit growth. In the case of *Streptococcus thermophilus*, due to the opacity of the media, 200uL aliquots of the overnight MICs were plated onto Milk Tomato Juice Yeast Extract Agar to observe growth compared to the positive control.

Controls

It is important to have several controls performed simultaneously for comparison and analysis.

Control 1—Negative control of sterile media placed into separate tube at time of testing to show aseptic technique were demonstrated.

Control 2—Positive control of sterile media inoculated with the same culture other tubes were inoculated with at the time of testing.

Control 3—Tests were performed in triplicate to verify results

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4. Method

Ten tubes were labeled from 1-10 with adhesive labels.

Protocol for Broth Macrodilution Method

Tube 1 have 1ml of antimicrobial and no media.

Tube 2 have 1ml of antimicrobial + 1ml of media, then vortexed

Tube 3 have 1ml of media + 1ml of the tube 2 mix added, then vortexed

Tube 4 have 1ml of media + 1ml of the tube 3 mix added, then vortexed

Tube 5 have 1ml of media + 1ml of the tube 4 mix added, then vortexed

Tube 6 have 1ml of media + 1ml of the tube 5 mix added, then vortexed

Tube 7 have 1ml of media + 1ml of the tube 6 mix added, then vortexed

Tube 8 have 1ml of media + 1ml of the tube 7 mix added, then vortexed. 1ml is removed and discarded.

Tube 9 positive control so it is only media plus culture, no antimicrobial.

Tube 10 negative control so it has only sterile media and is not inoculated with any culture.

After the tubes were set up according to protocol, bacteria in mid-log phase, diluted to 0.5 of McFarland standard was added in 1ml aliquots. Only tube 10 was not inoculated since it is the negative control.

Note: *Bifodobacteria* are strict anaerobes, so each inoculated tube required .04uL of Oxirase enzyme to bind all elemental oxygen and create an anoxic environment for growth. Also, the caps were double clicked to create a tight seal preventing oxygen from

altering the internal anoxic environment. They were still incubated at the same temperature.

The adjusted content of silver in each tube after dilution are as follows:

Tube 1—16ppm

Tube 2—8ppm

Tube 3—4ppm

Tube 4—2ppm

Tube 5—1ppm

Tube 6—0.5ppm

Tube 7—0.25ppm

Tube 8—0.13ppm

Tube 9—Positive control

Tube 10—Negative control

Media (see Appendix A for formulations)

***Lactobacillus* spp. require MRS Broth**

***Bifidobacteria* spp. require RCM**

***Streptococcus lactis* required TSB**

***Streptococcus thermophilus* required Tomato Skim Milk Yeast Broth**

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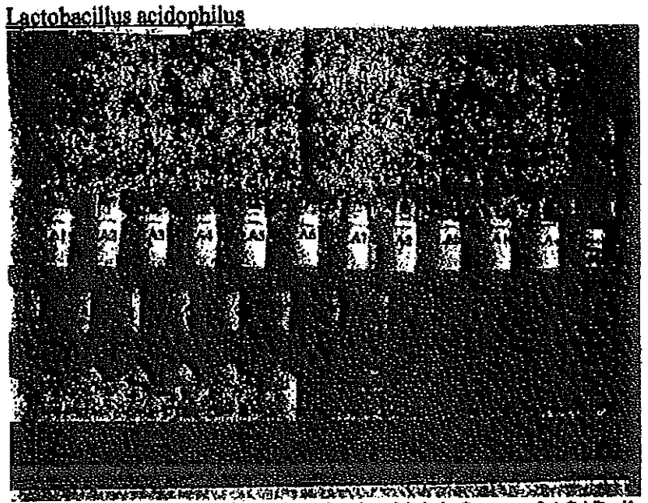
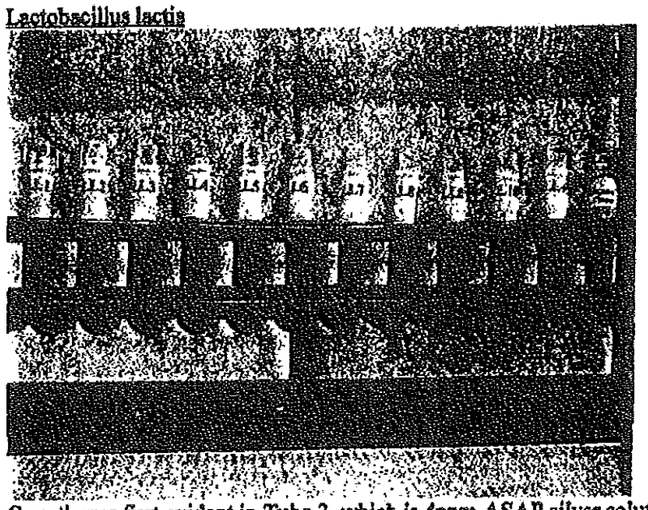
5. Results

Lactobacillus acidophilus



The first growth was evident in tube 4, which is 2ppm of ASAP silver solution.

Lactobacillus lactis



Growth was first evident in Tube 3, which is 4ppm ASAP silver solution.

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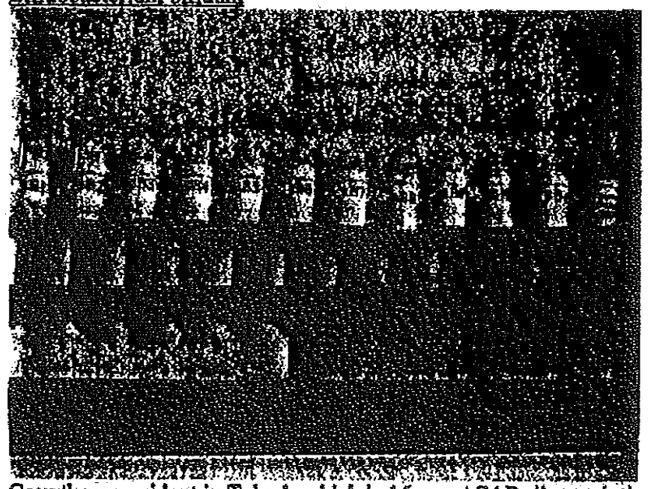
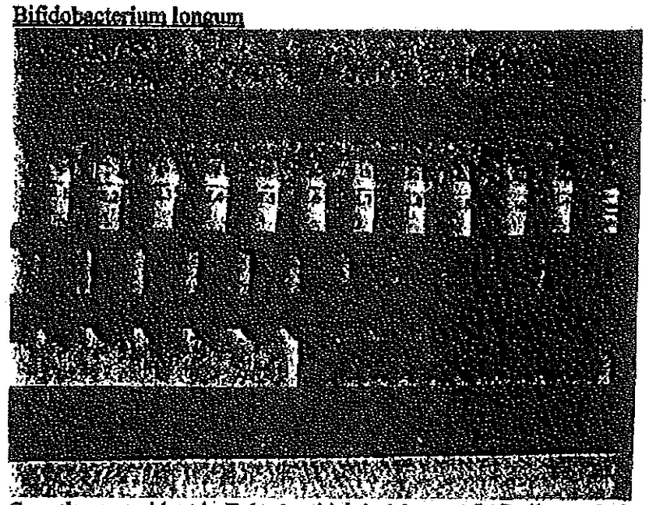
Bifidobacterium bifidum



Growth was evident in Tube 1, which is 16ppm ASAP silver solution.

Bifidobacterium longum



Growth was evident in Tube 1, which is 16ppm ASAP silver solution.

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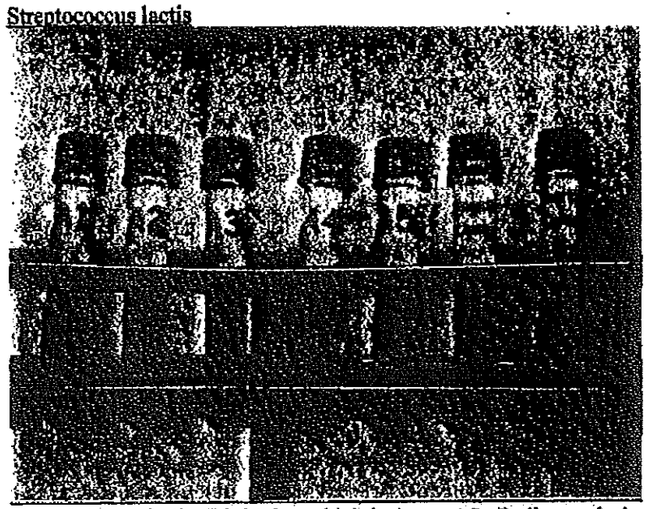
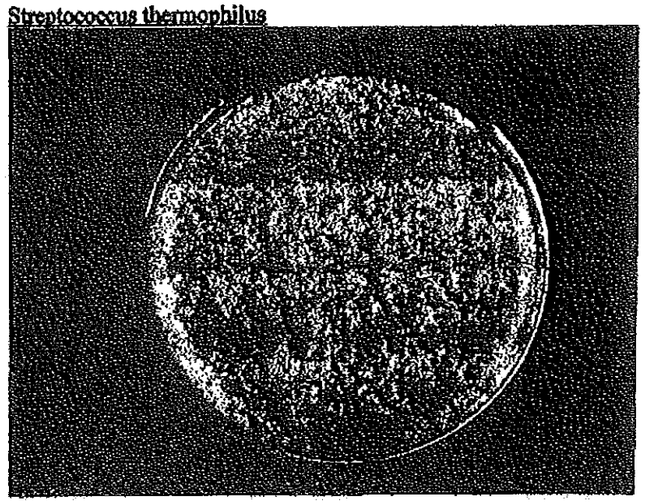
Streptococcus lactis



Growth began in the third tube, which is 4ppm ASAP silver solution.

Streptococcus thermophilus



Due to the nature of the media, Tomato juice Skim milk Yeast extract Agar, photographic evidence cannot accurately indicate growth. Visual inspection of plates after 24 hours of incubation and compared with positive control indicated that growth was first evident on plate 5 taken from Tube 5, which is 1ppm ASAP-AGX-32 silver solution.

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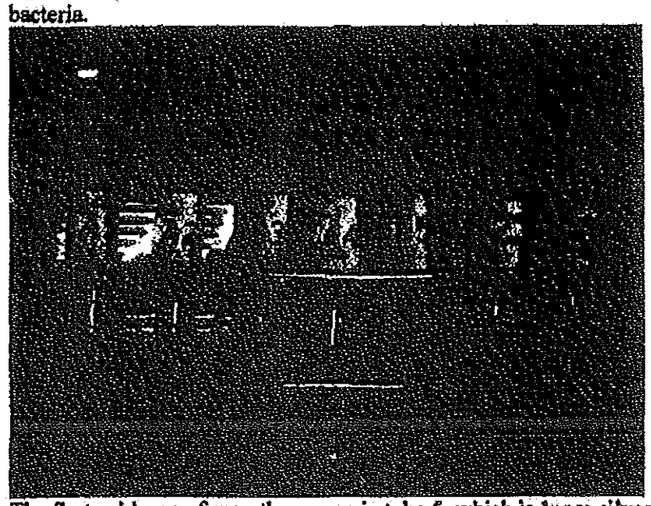
Salmonella typhimurium—causative agent of food poisoning called salmonellosis.
A comparison of the results of MIC testing of probiotics to a known pathogen demonstrated that the antibacterial efficacy of silver solution against probiotics is significantly less than observed in tests with the same silver solution against pathogenic bacteria.



The first evidence of growth appears in tube 5, which is 1ppm silver solution.

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6. Conclusion

Results obtained from MIC testing of probiotics with ASAP-AGX-32 silver solution in comparison to pathogenic microorganisms previously tested suggest that probiotics such as *Bifidobacteria* aren't affected at all by exposure to concentrations of 16ppm ASAP silver solution, whereas pathogenic bacteria are killed at concentrations as low as 2ppm. *Lactobacilli* are killed at concentrations of silver solution between 4 and 8ppm, but not at 2ppm. Silver solution concentrations greater than ~5ppm are bactericidal to *Streptococcus lactis*, and concentrations greater than ~2ppm are bactericidal to *Streptococcus thermophilus*.

We can understand from these results that strictly anaerobic bacteria such as *Bifidobacteria* aren't harmed by the ingestion of silver solution to treat bacterial infection, and *Lactobacilli* are minimally effected in comparison to the pathogens targeted by ASAP silver solution. It can therefore be concluded that the consumption of probiotics in conjunction with ASAP silver solution would be beneficial to the health of ill and healthy people alike.

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Appendix A—Media Formulation

M.R.S. Broth (de Man, Rogosa, Sharpe)
Peptone 10g/L
'Lab-Lemco' Powder 8g/L
Yeast extract 4g/L
Glucose 20g/L
'Tween' 80 1mL
Di-potassium hydrogen phosphate 2g/L
Sodium acetate trihydrate 5g/L
Tri-ammonium citrate 2g/L
Magnesium sulphate septahydrate 0.2g/L
Manganese sulphate quatrahydrate 0.05g/L
pH 6.2

Reinforced Clostridial Medium (RCM)
Yeast extract 3g/L
'Lab-Lemco' powder 10g/L
Peptone 10g/L
Soluble starch 1g/L
Glucose 5g/L
Cysteine hydrochloride 0.5g/L
Sodium chloride 5g/L
Sodium acetate 3g/L
Agar 0.5g/L
pH 6.8

Tryptic Soy Broth (TSB)
Pancreatic digest of Casein 17g/L
Papaic digest of soybean meal 3g/L
Dextrose 2.5g/L
Sodium chloride 5g/L
Dipotassium phosphate 2.5g/L
pH 7.3

Tomato Juice Skim Milk Yeast extract Broth
100mL of Tomato juice extracted from canned tomatoes through centrifugation
100g/L Skim Milk (we used Carnation)
Yeast extract 5g/L
1L of distilled water
pH 7

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