PN-ABC-888

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/30/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C7-111
1st pd. (3 mo) ~~First~~ year Report

Attachment

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STATE OF ISRAEL - מדינת ישראל

PN ABC-873

MINISTRY OF AGRICULTURE, KIMRON VETERINARY INSTITUTE, BEIT DAGAN

C 7 - 111

First period (3 months) - First year Report

Bacterial and Leucocytes Contents and Enzymes Levels in Milk as Methods for the Determination of Milk Quality and for the Detection of Mastitis

In the first 3 months of the research, following a short organization, the following experiments were initiated and conditions have been established.

a) Growing bacteria to be tested: E. Coli and S. Aurus. The bacteria were kept on agar slants in refrigiration. Prior to the testing, the bacteria were removed from the slant and grown in a broth to give a yield of 10^7 - 10^8 bacteria/ml.

The following biochemical tests were conducted:

1) TTC (triphenyltetrazolium Chloride).
The test is based on the principle that reduction of the tetrazolium salt by the bacteria yields a red-purple color, which could be early detected and measured. Testing fresh S. aurus bacteria or E. coli in their growing media, gave a clear positive result for the bacterial presence. Addition of bacteria to milk gave a clear positive result as well. Testing Mastitis milk gave a strong positive reading as well.
These preliminary studies revealed some difficulties concerning the detection of bacteria by the TTC test. Whenever the bacteria were frozen prior to its testing, or diluted with saline, the formed color was significantly reduced. Since the formed color in this test is very clear, the test, if proper analyzing conditions are established could be suitable for a stick test. This is one of the topics scheduled to be continued in the next stage.

2) <u>Nitrates</u> - using the Griess Nitrites test, showed the test to be potentially usefull for detecting bacteria in milk. Positive readings with S. aurus and E. coli suspended in their growing media or milk were obtained. Unlike the TTC test, no effect of dilution by saline or by freezing and thawing was seen. it is planned to evaluate a wider range of bacteria found in milk.

3) <u>Activation of nitrocellulose, nylon and cellulose</u> filter and membranes was done with cyanobromide or isonitrile.
Preliminary trials were run to evaluate the effect of proteins (antibodies) concentrations and duration of incubation on the binding to the activated membranes.
Protein concentrations ranging from 0.1-5.0 mg/ml and at incubation times of 2-24 hours were tried.
Good results were obtained with 0.1 mg protein/ml and incubation of 2 hours.

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4) Preparation of polyclonal antibodies against E. coli (k-99). This was obtained from the colostrum of immunized mothers, following fractionation with ammonium sulfate and dialysis.

Research Plans for the Next Stage

1) Improve test conditions for the TTC and nitrits tests for bacterial presence in milk.

2) Establish the optimal conditions for protein bindings to the ELIZA strip and compare the results to those obtained with plate ELIZA.

3) Evaluate the specificity and cross reactivity of E. Coli and S. Aurus.

4) Evaluate additional enzymes from bacterial origin as potential useful test.

5) During this 3 months period the collaborating Scientist from Sri-Lanka will come to the laboratory in Kimron Veterinary Institute (Israel).