extremity of the tail are due to such flaring out of the filament exposed by the breaking of the end piece, although in some cases such a break must have occurred very near the tip of the tail.

The fact that the filament and the extreme tip of the tail are composed of individual fibrils has been observed as early as 1886 and pictured by Ballowitz.[4] Since then this information has been disregarded by workers in the field of sperm morphology. Ballowitz calls these fibers the "Elementar-fibrillen" and ascribes to their presence the ability of sperm to move. He states, however, that in both birds and mammals the number of fibrils varies between 2 and 4. The electron microscope reveals that there are actually many more.

In stained preparations this brush phenomenon is never observed, possibly because the stain encases the fibers, cementing them into a rigid cylinder, thus not permitting them to flare out. In such preparations the naked terminal piece is clearly seen to be continuous with the axial filament of the end piece. (Fig. 7.) Usually following staining the very tip end of the tail appears clearly tapered although not brushed.

In the chicken sperm the head appears very dense, an acrosome is seen and the tail ends in a mass of long delicate fibers of sub-light-microscope dimensions. In chicken sperm also the middle piece breaks easily and releases a mass of fibers. This suggests that in the cock as well as in the bull the axial filament is made up of a bundle of many fibers.

We could not confirm the observation of Seymour and Benmosche[5] that the sperm head (human) has a suction disc nor could we find any "joints" in its middle pieces of any of the sperm types studied.

*Summary.* Fresh, unstained and unfixed samples of sperm from many fertile bulls studied under the electron microscope have shown that the anterior portion of the sperm head is always enveloped by a protoplasmic cap which appears damaged or disappears altogether if sperm are stained or fixed. This suggests that, contrary to results obtained with the optical microscope, the protoplasmic cap is not a sign of immature or abnormal sperm but is typical of normal sperm when these are examined without being exposed to solvents usually present in stains.

The tails end in a brush consisting of many free and very long filaments. Breaks in the main or end pieces of the tail have also shown flared brushes which make it seem likely that the axial filament consists of a bundle of fine fibers rather than a single relatively thick thread.

[4] Ballowitz, E., *Arch. f. Mikr. Anat.*, 1888, **32**, 401.

[5] Seymour, F. I., and Benmosche, M., *J. A. M. A.*, 1941, **116**, 2489.

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## Utilization of Asparagus Juice in Microbiological Culture Media.

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This laboratory has a series of investigations in progress which relate to the possible usefulness of waste vegetable juices as microbiological culture media. The observation that asparagus-butt juice undergoes extraordinarily rapid microbiological spoilage, together with the interest now being shown in the production of antibacterial agents for use in the treatment of certain disease and wound infections, has led us to investigate the possibility of utilizing this juice as a medium for culturing some of the organisms that produce

antibacterial substances. Attention has also been given to the production of bacterial proteolytic enzymes.

The annual accumulation of asparagus waste (butt trimmings and culls) at packing houses and canneries in the United States has been estimated to range from 50,000 to 100,000 tons. This waste constitutes a major waste-disposal problem at asparagus processing centers. Marsh and Cruess[1] have previously recommended use of asparagus-butt juice to replace the brine solution used in canning asparagus.

Asparagus-butt juice appears to provide an excellent balance of nitrogen, sugar, and inorganic constituents for microbiological nutrition, though information concerning its precise chemical composition is rather meager. Analyses of various lots of juice prepared in this laboratory indicate that it contains approximately 4 to 5% solids, of which the major part is reducing sugar. The juice as pressed from fresh asparagus butts and completely clarified contains generally 0.07 to 0.13% total nitrogen.

*Tyrothricin production.* Asparagus-butt juice has been found to be suitable for the growth of *Bacillus brevis* and for the production of tyrothricin. Butt trimmings from both green and white asparagus that had been stored at 0°F for approximately 6 months were used as a source of juice. A cold-pressed juice was prepared by grinding the frozen butts in a meat grinder, followed by thawing and pressing in a hydraulic cider press. A hot-pressed juice was prepared by allowing the butts to thaw and then steaming 20 minutes at 100°C in a vegetable blancher. The steamed material was also pressed in a hydraulic cider press.

The pressed juices were clarified by filtration through a Mandler filter of medium porosity or through paper in a Buchner funnel after addition of diatomaceous filter aid. It was necessary to heat the cold-pressed juice several minutes at 100°C before filtration to precipitate heat-coagulable material and thereby to insure complete clarity after steam sterilization. The clarified juices were distributed in 50-ml portions in 250-ml Erlenmeyer flasks and sterilized by autoclaving for 20 minutes at 10 pounds of steam pressure. After sterilization the amount of normal sodium hydroxide required to adjust the pH to 7.5 was added to each flask aseptically.

The culture of *Bacillus brevis* used was the B. G. strain obtained from Dr. René Dubos at the Rockefeller Institute for Medical Research, New York City. The inoculum was prepared by making a transfer from a stock culture of beef agar to a beef agar slant and incubating 24 hours at 35°C. Transfer was then made with sterile water to a 25-ml portion of asparagus-butt juice which was then incubated 24 hours at 35°C. The flasks containing the 50-ml portions of juice media were then inoculated with 0.5 ml each of the inoculum and incubated for 2, 4, 6, and 10-day intervals at 35°C. Flasks were harvested in triplicate for tyrothricin assay after each of the indicated incubation periods.

The tyrothricin contents of the cultures were determined by a hemolytic method recently developed in this laboratory.[2] A standard preparation of tyrothricin was used as a control. The fractionation procedure of Dubos and Hotchkiss[3] with slight modification was used to determine actual recoverable yields of tyrothricin. The results obtained by the hemolytic method are shown in Table I.

A maximum of 2.0 g of tyrothricin per liter of culture medium was indicated in the hot-pressed green asparagus-butt juice after 10 days' incubation. This value was not checked by actual tyrothricin recovery. However, experience gained subsequently indicates that in most cases 90% or more of the tyrothricin found in asparagus juice cultures by the hemolytic assay method may be recovered by isolation.

Dubos and Hotchkiss[3] reported that yields on tryptone media may vary considerably, ranging from 0.02 to 0.50 g of tyrothricin per liter of culture medium. Stokes and Wood-

[1] Marsh, G. L., and Cruess, W. V., *Fruit Prod. J. and Am. Vinegar Industry*, 1942, **21**, 333.

[2] Dimick, Keene, *J. Biol. Chem.*, 1943, **149**, 387.

[3] Dubos, René J., and Hotchkiss, Rollin D., *J. Exp. Med.*, 1941, **73**, 629.

TABLE I.
Yields of Tyrothricin Obtained from Cultures of *Bacillus brevis* Grown in Asparagus Butt Juice Media.

| Source of juice | Preparation of juice | Yields of tyrothricin by hemolytic assay.* Length of time of incubation | | | |
|---|---|---|---|---|---|
| | | 2 days g/l | 4 days g/l | 6 days g/l | 10 days g/l |
| White asparagus | Cold pressed | 0.48 | 0.57 | 0.95 | 1.23 |
| " " | Hot " | 0.47 | 1.00 | 1.27 | 1.68 |
| Green " | Cold " | 0.56 | 1.23 | 1.39 | 1.63 |
| " " | Hot " | 0.62 | 1.31 | 1.67 | 2.01 |

* Hemolytic assays by Dr. Keene Dimick. Each value is an average of 3 determinations.

ward[4] reported yields ranging from 100 mg to a maximum of 300 mg per liter in a variety of media under submerged conditions of culturing.

*Antibacterial substance produced by Bacillus subtilis. Bacillus subtilis* was observed to form an usually heavy pellicle after 24 hours' incubation when grown on asparagus-butt juice. The strain used was No. 231 of the culture collection of N. R. Smith of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Washington, D.C. While this study was in progress, Katznelson[5] reported that a substance toxic to microörganisms was produced by this organism.

A hot-pressed juice previously concentrated under vacuum at low temperature (25 to 30° C) to 9.4% soluble solids content was used as a medium to investigate antibacterial activity of *B. subtilis*. The juice was sterilized by Seitz filtration, distributed in 50-ml portions in sterile 250-ml Erlenmeyer flasks and the pH was adjusted to 7.0 with sterile normal sodium hydroxide. A suspension of *B. subtilis* in 10 ml of water washed from a beef agar slant after 24 hours' incubation at 30° C was used for inoculation.

The cultures were incubated at 35° C for 24 hours and harvested in triplicate. The pH of the harvested culture liquors was adjusted to 2.5 with hydrochloric acid, followed by sterilization at 10 lb steam pressure for 10 minutes. The 3 replicates were combined and the antibacterial activity determined by means of a serial dilution method against a number of test organisms including *Micrococcus conglomeratus* (Merck's MY strain), *Staphylococcus aureus* (U. S. Food and Drug Administration Strain No. 209), *Lactobacillus casei* (ATCC No. 7469), and three plant pathogens, *Erwinia amylovora, Phytomonas juglandis,* and *Phytomonas michiganensis,* obtained from Dr. P. A. Ark of the University of California, Berkeley, California.

The test medium used for determining antibacterial activity against all of the test organisms except *S. aureus* was a yeast-extract glucose broth. Beef broth was used in the case of *S. aureus*. The tubes containing the serial dilutions were incubated 24 hours at 30° C for all test organisms except for *S. aureus,* in which case the temperature was 35° C. A solution of tyrothricin was used as a standard in appropriate dilutions against the same test organisms. The results are shown in Table II.

Growth was measured by recording the difference in turbidity readings between the inoculated tubes and the uninoculated controls. A Klett-Summerson colorimeter was used for this purpose.

The data indicate the comparatively high inhibiting action of the *B. subtilis* cultures against all of the test organisms except the two Gram-negative plant pathogens, *E. amlyovora* and *P. juglandis*. The exceptionally high activity against *Phytomonas michiganensis* seems noteworthy. A gradual adaptation of this plant pathogen to the action of the antibiotic substance appears to take place, though not to the same degree as with *S.*

[4] Stokes, J. L., and Woodward, C. R., Jr., a paper presented at the joint meeting of the New Jersey and New York sections of the Society of American Bacteriologists in New York City, December 29, 1942.

[5] Katznelson, H., *Canadian J. Res.*, 1942, **20**, 169.

TABLE II.
Comparison of the Antibacterial Activity of a *Bacillus subtilis* Culture with That of a Tyrothricin Standard Against Various Test Organisms.

| Test organisms | Growth of test organisms and dilution ratios | |
|---|---|---|
| | *B. subtilis* culture | Tyrothricin standard (0.5 g per liter) |
| *Micrococcus conglomeratus* | —1:10,240; 90%* at 1:20,480 | —1:2,560; 90% at 1:5,120 |
| *Staphylococcus aureus* | —1:640; 60% at 1:1,280 | —1:80; 40% at 1:160 |
| *Lactobacillus casei* | —1:40,960; 50% at 1:81,920 | —1:640; 80% at 1:1,280 |
| *Erwinia amylovora* | —1:80; 40% at 1:160 | —1:20; 60% at 1:40 |
| *Phytomonas juglandis* | —1:80; 15% at 1:160 | —1:20; 50% at 1:40 |
| *Phytomonas michiganensis* | —$1:10^{15}$† | —1:640; 40% at 1:1,280 |

* Percentages indicate estimates of growth based on turbidity readings. Negative signs indicate highest dilution ratios at which growth of the test organism was completely inhibited.
† Highest dilution tested; growth obtained at lower dilutions after longer incubation (See text).

*aureus*. Complete inhibition of *P. michiganensis* was obtained at a dilution of $1:10^{12}$ after 4 days' incubation, which was further reduced to the order of 1:5,000,000 after 6 days.

*Antibacterial substances produced by fungi.* Preliminary investigations of the suitability of media prepared from asparagus-butt juice indicated that both *Penicillium notatum* and *Penicillium citrinum* can be grown successfully with the production of good antibacterial activity in the culture liquors. Yields of citrinin in excess of 2 g per liter of culture medium can readily be obtained after 10 days' incubation at 30°C. These results compare favorably with those obtained by Raistrick and Smith[6] using a synthetic medium. Yields of penicillin have not been determined.

*Proteinase production.* Asparagus-butt juice has also been found to be suitable for the production of proteolytic enzymes, particularly by the *scaber* strain of *Bacillus subtilis* obtained from Dubos. Only minor supplementary treatment of the juice appears to be necessary for obtaining satisfactory yields. The proteinase obtained is similar to commercially produced bacterial proteolytic enzymes, and it is probable that asparagus concentrates can be used to replace more expensive industrial media.

*Summary.* The suitability of press juices prepared from waste asparagus butt trimmings as a source of the major components of media for culturing certain microörganisms of potential commercial importance has been indicated. The use of such media for the production of certain antibacterial substances and proteolytic enzymes appears to have particular promise.

[6] Raistrick, Harold, and Smith, George, *Chemistry and Industry*, 1941, **60**, 828.