yielded dextran added. The combination of all three factors yielded dextran with a relative viscosity as low as 1.6. The decrease in viscosity occurred in an orderly manner.

For the recovery of dextran of clinical size, only the addition of dextran was of any value. A yield of 34.6 per cent of the recoverable dextran was attained, which was more than twice the recovery achieved by means of acid hydrolysis.

In conclusion, a procedure for achieving a high dextran yield of molecular weight 75,000 ± 25,000 by direct bacterial synthesis was successfully developed.

REFERENCES

Enhancement of Antibiotic Production by the use of Sea Water Media

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Sea water is a most appropriate environment for living cells, since it contains all of the chemical elements essential to growth and maintenance of plant and animal protoplasm. It is a buffered solution and contains probably all of the chemical elements although only about 50 have so far been detected. The ratios of the major salts to each other, and usually their total concentration also, are strikingly similar in sea water and in body fluids of plant and animal cells (Sverdrup, Johnson and Fleming, 1942).

In the production of antibiotic substances by streptomycetes and molds, trace elements are necessary for good yields but the precise relationships are not clear (Chesters and Rollinson, 1951; Foster et al., 1943; Jarvis and Johnson, 1950; Johnstone and Waksman, 1948; Le Page and Campbell, 1946; Moyer and Coghill, 1946; Principe and Thornberry, 1952; Reynolds and Waksman, 1948; and Woodruff, 1947).

The ratio or balance of these elements to one another is very important. The stimulatory effect exerted by one element may be cancelled by an excessive amount of another (Johnstone and Waksman, 1948). Conversely the toxic effect of a certain element may be antagonized by the presence of a proper amount of another.

Culture media for antibiotic production are generally prepared with regard to trace elements. These may possibly contain sufficient quantities of trace elements but not necessarily in optimum balanced concentration. On the other hand, certain media containing complex constituents such as beef extract, may contain excessive or toxic quantities rather than insufficient concentrations of trace elements (Chesters and Rollinson, 1951).

In screening large numbers of organisms for antibiotic activity, it is desirable to have a medium that will be properly supplemented with trace elements. Accordingly sea water was used as a constituent of
SEA WATER MEDIA EFFECT ON ANTIBIOTIC PRODUCTION

Fig. 1

Fig. 2

Fig. 3

Fig. 4

SW = Sea Water; TW = Tap Water; DW = Distilled Water.
media for the cultivation of antibiotic-producing streptomycetes.

**Materials and Methods**

With the exception of *Streptomyces griseus*, the organisms studied were random isolates of species of *Streptomyces* from various soils which exhibited antagonistic properties. No attempt was made to identify the organisms.

*Test organism.* *Micrococcus pyogenes* var. *aureus* A.T.-C.C. 6538 was used as the test organism for antibiotic activity. It was maintained in nutrient broth and transferred daily.

*Sea water.* The sea water was collected at a distance of at least two miles from shore and appeared to be visibly clear and free of particulate debris. It was filtered through paper, then subjected to vigorous aeration for six days to oxidize any soluble organic matter and to stabilize the solution. The loss in volume was replaced with distilled water. The sea water was then stored at room temperature in tightly capped bottles and used as needed.

*Basal medium for antibiotic production.* The basal medium used throughout this study had the following composition: Glucose, 10 gm; peptone, Difco, 5 gm; yeast extract, Difco, 3 gm; water, to make 1000 ml. The water consisted of (1) distilled water, (2) tap water, (3) distilled water with various concentrations of NaCl, and (4) distilled water with various concentrations of sea water.

In all cases, except where 100 per cent sea water was studied, the ingredients were dissolved in a minimal amount of distilled water in order that sea water could be added to any desired concentration. Adjustment to proper volume was then made with distilled water. The pH was 6.5 to 6.7 without adjustment. The media were distributed in 125 ml quantities into 500 ml Erlenmeyer flasks, and sterilized at 15 lb pressure for 20 min. A slight precipitate developed in the 100 per cent sea water medium after sterilization; the media containing less sea water remained clear after sterilization.

*Preparation of inoculum.* The inoculum was prepared by seeding a flask of basal medium, containing distilled water without sea water, with a spore suspension of the organism, then agitating the flask on a Burrell wrist action shaking machine for 24 hr at 28 C.

*Antibiotic production.* The flasks containing the different sea water media were inoculated with 1 ml of a 24-hr vegetative growth culture of the streptomycete. Samples were removed aseptically after 72, 96, 120, and 144 hr of incubation. The cell material was removed by centrifugation and the supernatant assayed for antibiotic activity by a combination of the methods of McMahon (1944) and Joslyn and Galbraith (1950).

*Assay medium.* The assay medium contained the following: Peptone, Difco, 5 gm; yeast extract, Difco, 3 gm; glucose, 3 gm; distilled water, 1000 ml. The medium was sterilized in the autoclave at 15 lb pressure for 20 min.

*Method of assay.* One ml assay broth was added to each of a series of tubes. To the first tube was added 1 ml of the antibiotic-containing supernatant and mixed. One ml from the first tube was transferred to the second tube and mixed. One ml from the second tube was transferred to the third tube. This was continued until a series of two-fold dilutions was prepared with 1 ml in each tube.

An 18-hr culture of *Micrococcus pyogenes* var. *aureus* in nutrient broth was diluted 8 times in assay medium and 0.2 ml of the dilution added to each 10 ml of assay medium contained in a flask. To each of the antibiotic dilutions was added 9 ml of this inoculated assay medium by means of a Brewer automatic pipette. The tubes were incubated at 37 C for about 51/2 hr, then placed in an ice bath to stop growth. After 10 min, 1 drop of 1 per cent formalin was added to each tube, and readings made for turbidity in a Cenco-Sheard-Sanford photometer containing a No. 87309 B green filter.

*Results*

The results are plotted on graphs 1 to 4 inclusive. The ordinates represent the percentage of light transmission in the inoculated broth and the abscissae the dilutions of the fermentation supernatants.

The age of the fermentation sample used for analysis, as well as the code number of the organism, is indicated on each graph. The graphs shown for the individual organisms represent samples taken at a time when antibiotic production had reached its peak, although samples taken at other ages showed the same pattern.

Ten different organisms of the genus *Streptomyces* were studied to show the effect of sea water on antibiotic production, but the results of only four are presented here. One graph is shown for each organism. It can be seen that sea water was superior to (1) distilled water, (2) tap water, and (3) distilled water with NaCl added. Of the ten organisms studied, only one gave slightly better antibiotic production in NaCl than in sea water. This is shown with organism SG MA13. Tap water and distilled water were always inferior to sea water.

*Discussion*

The results show that organisms of the genus *Streptomyces* vary considerably in their reaction to sea water, some producing more antibiotic than others. In general, sea water is superior to sodium chloride, and considerably more so than either tap water or distilled water. The fact that sodium chloride is inferior to sea water when present in equivalent concentrations, would indi-

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cate that trace elements supplied by the latter play some important role in the metabolism of the organisms.

This noticeable difference in antibiotic production shows that a medium prepared with distilled water or tap water and not properly supplemented with trace elements, if used in a survey, would give only a small percentage of organisms actually capable of producing antibiotic substances. Sea water appears to be a means of overcoming this deficiency by providing a balanced trace element additive.

No attempt was made to determine the most effective concentration of sea water to be used. As seen from the graphs, this would vary for each organism, and, for the purposes of this study, it was not considered important, although according to the data presented, the optimum is generally around 40 per cent or perhaps higher.

Likewise, no attempt was made to compare various types of culture media with the addition of sea water. Since only one basal medium was used in this study, this leaves open the question of the effect of various media constituents combined with sea water on antibiotic production by a group of streptomycetes.

**Summary**

It has been shown that sea water is superior to sodium chloride, tap water, or distilled water for antibiotic production by a group of streptomycetes. This would indicate that certain trace elements present in sea water play an important role in the metabolism of the organisms.

**REFERENCES**


