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Isolation of Cellulolytic Actinomycetes from Marine Sediments

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The cellulolytic activity of 36 actinomycetes strains isolated from marine sediments was investigated by the cellulose-azure method. Approximately 50% of the isolates exhibited various degrees of cellulolytic activity.

For a number of years, most screening programs for microorganisms with cellulolytic activity were restricted mainly to molds (2, 3, 6–8). The search for new and different cellulose-degrading microorganisms was increased, and new species have been isolated from various natural habitats. In recent years, the possibility of obtaining high yields of commercially useful cellulase from actinomycetes has attracted the attention of researchers, and several mesophilic and thermophilic strains have been isolated for this purpose (4, 5, 12). However, no example of cellulolytic activity from marine actinomycetes has been reported. In the present study, 36 actinomycetes strains were isolated from marine sediments and examined for their possible abilities to attack cellulose.

Four marine sediment samples were collected with sterile containers at two places about 10 m deep in La Coruña Bay (Spain). All of the closed containers were brought back to the laboratory within one-half day of collection. On arrival at the laboratory, they were shaken for 1 h on a rotary shaker at 250 rpm to disperse the samples. After that, samples were properly diluted and spread over both a starch-casein medium (13) and a K medium (9). After 7 days of incubation at 28°C, colonies of suspected actinomycetes were selected. Stock cultures of the isolates were maintained on yeast extract-malt extract agar (13) slants and were later tested for cellulolytic activity.

Cellulolytic activity was examined by the cellulose-azure method described by Smith (10) for the screening of cellulolytic fungi. This method was combined with the gravimetric determination of cellulose digestion as follows. Isolated actinomycetes were grown in a *Trichoderma viride* medium (11) with ball-milled cellulose as the only carbon source. The pH was adjusted to 7.3. The cultures were incubated for 7 days at 28°C on a rotary shaker run at 300 rpm in 50-ml Erlenmeyer flasks with 15 ml of medium in each flask. The flasks were inoculated with an abundant cell mass from stock cultures. An uninoculated flask was used as the control. Digestion of cellulose was calculated by comparing the weight loss of the inoculated flasks with that of the control flask after both samples were dried to a constant weight. The degree of digestion was expressed as a percentage of the latter.

In both methods, *T. viride* ATCC 13631 was used as a cellulolytic organism reference.

The cellulose-azure method may be perfectly well applied for screening cellulase-producing actinomycetes. The simplicity of this assay makes it useful for screening the cellulolytic capacity of large numbers of these microorganisms. Results of cellulolytic activity determinations indicate that of the 36 strains tested, 19 (>50%) showed enzymatic degradation of cellulose (Table 1). Six isolates, which scored negative on the cellulose-azure test after 7 days of incubation, digested in that time over 80% of the supplied cellulose when grown in shaken flasks. However, this does not constitute a serious handicap for the utilization of the cellulose-azure method and may be easily explained by the different culture conditions used in the two assays. The submerged cultures allow a faster metabolism, probably due to better contact of cells with the cellulose fibers and to a greater release of cellulases by the Tween 80 incorporated into the liquid medium; in addition, the cellulose-azure tubes were surface inoculated with one loop of spores, whereas the shaken flasks were inoculated with an abundant cell mass. It is apparent also that the degree of digestion reached by several isolates was near 100%. By microscopic examination, it was confirmed that the strains S20, S22, and S27, which digested over 95% of the cellulose after 7 days of incubation, were completely lysed in that time. The other strains showed only traces of mycelia, and no true pellets were observed at any time.

By means of the Becker et al. (1) technique

TABLE 1. Screening of marine actinomycetes with cellulolytic activity[a]

Strain no.	Cellulose-azure test result by the following day of incubation:			% Cellulose digestion (7 days fermentation)
	7	14	21	
S1	−	−	−	NT
S2	−	−	−	NT
S3	−	−	−	NT
S4	−	+	++	83.4
S5	−	−	−	NT
S7	−	−	++	NT
S9	−	−	−	NT
S11	−	−	−	NT
S12	−	−	+	NT
S14	−	−	−	NT
S16	−	+	++	83.5
S18	−	−	−	NT
S19	−	−	−	NT
S20	+	++	+++	100
S21	−	+	+	NT
S22	+	++	+++	99.5
S23	−	−	−	NT
S24	+	++	+++	NT
S25	−	−	++	34.4
S26	−	−	−	NT
S27	−	++	++	96.8
S28	−	++	++	92.9
S29	−	−	−	NT
S30	−	+	++	81.2
S31	−	−	−	NT
S32	−	−	++	38.5
S34	−	−	−	NT
S35	−	++	++	95.8
S36	−	−	++	NT
S37	−	−	++	NT
S38	−	−	−	NT
S40	−	+	++	40.1
S41	−	−	−	NT
S42	−	−	−	NT
S43	−	−	+	NT
S44	−	−	+	37.8
T. viride ATCC 13631	+	++	+++	46.5

[a] Symbols: −, Negative; +, weakly positive; ++, positive; +++, strongly positive; NT, not tested.

and microscopic examination, all isolates with cellulolytic activity were identified as belonging to the genus *Streptomyces*.

The actinomycetes collected from La Coruña Bay were tested for growth in 3.5% NaCl. All isolates in this experiment were able to grow in media containing this salt concentration. They may represent an autochthonous marine flora, or, since the samples were taken in the littoral zone, they are more likely to be land organisms that were carried into the sea and which were preadapted to the salinity of the seawater and sediments. In any case, in view of the high percentage of cellulolytic isolates, we think a marine environment could be a source as important for the discovery of new cellulolytic microorganisms as a land environment. Besides, since the environmental conditions of the sea are extremely different from land conditions, microorganisms in the sea may produce cellulases with optimal activity at lower temperatures and different pHs than cellulases from land organisms.

We conclude that this report is the first to demonstrate a cellulolytic activity in actinomycetes isolated from marine sediments. Further work is in progress to study the enzymatic complex of some marine isolates in this experiment.

LITERATURE CITED

1. **Becker, B., M. P. Lechevalier, R. E. Gordon, and H. A. Lechevalier.** 1964. Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. Appl. Microbiol. **12**:421–423.
2. **Enari, P., and A. F. Markkanen.** 1977. Production of cellulolytic enzymes by fungi. Adv. Biochem. Eng. **5**:1–24.
3. **Fujio, Y., and M. Moo-young.** 1980. Isolation of cellulolytic fungi and some properties of isolated fungi for cellulose biodegradation. J. Gen. Appl. Microbiol. **26**:37–44.
4. **Hägerdal, B. G. R., J. D. Ferchak, and E. K. Pye.** 1978. Cellulolytic enzyme system of *Thermoactinomyces* sp. grown on microcrystalline cellulose. Appl. Environ. Microbiol. **36**:606–612.
5. **Ishaque, M., and D. Kluepfel.** 1980. Cellulase complex of a mesophilic Streptomyces strain. Can. J. Microbiol. **26**:183–189.
6. **Kane, B. E., and J. T. Mullins.** 1973. Thermophilic fungi in a municipal waste compost system. Mycologia **65**:1087–1100.
7. **Mandels, M.** 1975. Microbial sources of cellulases. Biotechnol. Bioeng. Symp. **5**:81–105.
8. **Mandels, M., and E. T. Reese.** 1964. Fungal cellulases and the microbial descomposition of cellulosic fabric. Dev. Ind. Microbiol. **5**:5–19.
9. **Okazaki, T., and Y. Okami.** 1972. Studies on marine microorganisms. II. Actinomycetes in Sagami Bay and their antibiotic substances. J. Antibiot. **25**:461–466.
10. **Smith, R. E.** 1977. Rapid tube test for detecting fungal cellulase production. Appl. Environ. Microbiol. **33**:980–981.
11. **Sternberg, D.** 1976. Production of cellulase by Trichoderma. Biotechnol. Bioeng. Symp. **6**:35–53.
12. **Su, T. M., and J. Paulavicius.** 1975. Enzymatic saccharification of cellulose by thermophilic Actinomyces. Appl. Polym. Symp. **28**:221–236.
13. **Williams, S. T., and T. Cross.** 1971. Actinomycetes. Methods Microbiol. **4**:295–334.