

## BACTERIA AND ACTINOMYCETES GROWING ON FLOPPY AND COMPACT DISCS UNDER AMBIENT CONDITIONS

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### Abstract

Floppy and compact discs gradually lose their quality. On closer examination deteriorated discs showed diverse microbial growth on their surfaces. Among the associated organisms 15 actinomycetes and 12 bacterial isolates were selected for detailed studies. The selected actinomycetes were *Streptomyces diastaticus*, *S. rimosus*, *S. cyaneus*, *S. olivaceoviridis*, *S. halstedii*, *S. fulvissimus*, *S. albidoflavus*, *S. exfoliates* and *S. griseoflavus* and the bacterial strains were *Bacillus lentus*, *B. circulans*, *B.adius*, *B. laterosporus*, *B. larvae* and *B. thuringiensis*. In addition, *Micrococcus sedentarius* was also isolated. Utilization of crushed disc materials was observed during this study, which was indicative of deterioration of floppy and compact discs.

### Introduction

In the quest for archiving important data and information, from ancient time men used different types of materials including stone, clay, leaves, barks, animal skins and so on, and in modern time on magnetic tapes, floppy disc, compact disc etc.

Floppy disc is made up of materials like mylar plastic (polyester and iron oxide), poly vinyl chloride etc. It is the advanced edition of magnetic tape. The constituent elements of compact disc are polycarbonate plastic, metallic layer, organic dye, lacquer etc. Seemingly, there is no nutrient element on its surface to support microorganisms to grow. But in the humid tropical environment, microorganisms are often found to grow on these materials and exploit their usability including that of Bangladesh. The biodegradation of different recalcitrant materials *viz.* glass (Khan and Khan 1977), magnetic tape (Khan and Karim 1996), polythene under natural (Khan *et al.* 2000) and laboratory conditions (Mumtaz *et al.* 2001) were reported. If a particular compound is added repeatedly to a microbial community, adaptation and faster rate of degradation can occur. These microbial communities could transform the molecules of recalcitrant materials as normal metabolic intermediates by using special enzymes and pathways (Prescott *et al.* 2002).

Floppy and compact discs are usually used to store important data and expected that these will last for a long time. But after a few days microorganisms are noticed on these materials which hamper the quality and functions of these goods. Due to their recalcitrant nature, it is not possible to observe the complete degradation process within a few months or not even a few years.

The present work was undertaken to isolate and study the microorganisms associated with floppy and compact discs. Experiments were also designed to examine the possible role of these organisms in the biodegradation process of these materials.

### Materials and Methods

Different types of floppy and compact discs were collected from different shops and residences. The collected floppy discs having brand names were mostly unusable. These were kept in plastic bag at room temperature for detailed studies. The collected discs were examined both under naked eyes and under microscope for microbial growth. Here phase contrast and fluorescence microscopy were helpful in revealing microbial growth.

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During the period of this work, July 2005 to December 2006, the minimum temperature was 15.7°C in December 2005 and the maximum was 33.7°C during April-May 2006. The prevailing humidity was minimum (48.2%) in March 2006 and maximum (80.1%) in August 2006.

Direct inoculation and enrichment culture techniques were used for the isolation of the microorganisms from the collected samples. Some floppy discs were buried under soil and after three months the discs were retrieved for observation. Pieces of retrieved floppies were inoculated in different media for growing the microorganisms. The enrichment culture was used to isolate organisms with rare capabilities (Prescott *et al.* 2002). So modified compact disc media were prepared using different layers of crushed CD and then CD with microbial growth was inoculated.

Normally, actinomycete is a slow grower. In the present study, different isolation media *viz.* oatmeal agar (Shirling and Gottlieb 1966), glucose asparagine agar (Waksman 1967), modified Bennett's agar (Iwasa *et al.* 1970), nutrient agar (NA) and nutrient broth (NB) media were used. Nystatin (50 µg/ml) was added in each plate to avoid fungal contamination (Williams and Davies 1965).

Bacteria were studied by using wet mount and stained smear techniques; while actinomycetes were observed by applying special techniques such as, whole mount, cover slip culture on solid medium (Kawato and Shinobu 1959), cover slip culture in liquid medium (Luedemann 1969) and impression.

Physiological and biochemical tests, as stated in the Bergey's manual Vol. 2 (Sneath *et al.* 1986) and Vol. 4 (Williams *et al.* 1989) were carried out and an attempt was made to identify organisms. Manual of Microbiological Methods (SAB 1957), Microbiological Methods (Collins and Lyne 1984) and Understanding Microbes (Claus 1995) were also consulted.

An experiment was designed to investigate whether the constituents of compact disc were utilized by the organisms or not. To carry out such investigation, crushed compact disc was added with tryptone yeast extract broth (ISP-1) and nutrient broth (NB) media. Each type of media had two sets - one set contained 1.5% crushed CD and another set with only diluted broth (control). Two isolates from compact disc *viz.* CDA-8 and CDA-13 were inoculated, respectively in ISP-1 and NB media. The result was observed after 28 days of incubation at room temperature.

## Result and Discussion

During the observations of floppy and compact discs, a number of the samples showed visible microbial growth as judged by eye inspection, while others revealed the presence of such organisms only under microscope. Two types of microorganism *viz.* bacteria and actinomycetes were isolated from these materials (Fig.1A-D). The selection of the isolates was made on the basis of their growth response and variation in colonial characteristics. From floppy disc, six actinomycetes and 28 bacterial colonies were isolated and out of these four actinomycetes and seven bacterial colonies were selected for detailed studies. In case of compact disc, 11 actinomycetes and five bacterial colonies were selected from 30 actinomycetes and 23 bacterial colonies. Purified bacterial colonies were maintained on nutrient agar slants and actinomycetes on tryptone yeast agar and yeast extract malt extract agar media.

Comparing the results with the standard description in the Bergey's Manual Vol. 2, the selected bacterial isolates were provisionally identified. Except isolate No. FB-4, all the bacterial isolates belonged to the genus *Bacillus viz.* *B. lentus* (CDB-1, CDB-8, FB-7), *B. circulans* (CDB-2), *B.adius* (CDB-5, FB-1, FB-20), *B. larvae* (FB-2), *B. laterosporus* (FB-5), *B. thuringiensis* (CDB-10, FB-16) while FB-4 belonged to *Micrococcus sedentarius*.

The selected actinomycetes isolates were provisionally identified with the help of Bergey's Manual Vol. 4. All the selected actinomycetes belonged to the genus *Streptomyces viz.* *S.*

*diastaticus* (CDA-1, CDA-4, CDA-5), *S. griseoflavus* (CDA-6), *S. cyaneus* (CDA-7), *S. olivaceoviridis* (CDA-8), *S. halstedii* (CDA-10, FA-1), *S. albidoflavus* (CDA-12), *S. fulvissimus* (CDA-13), *S. rimosus* (CDA-15, FA-2, FA-3, A-5) and *S. exfoliates* (CDA-18).

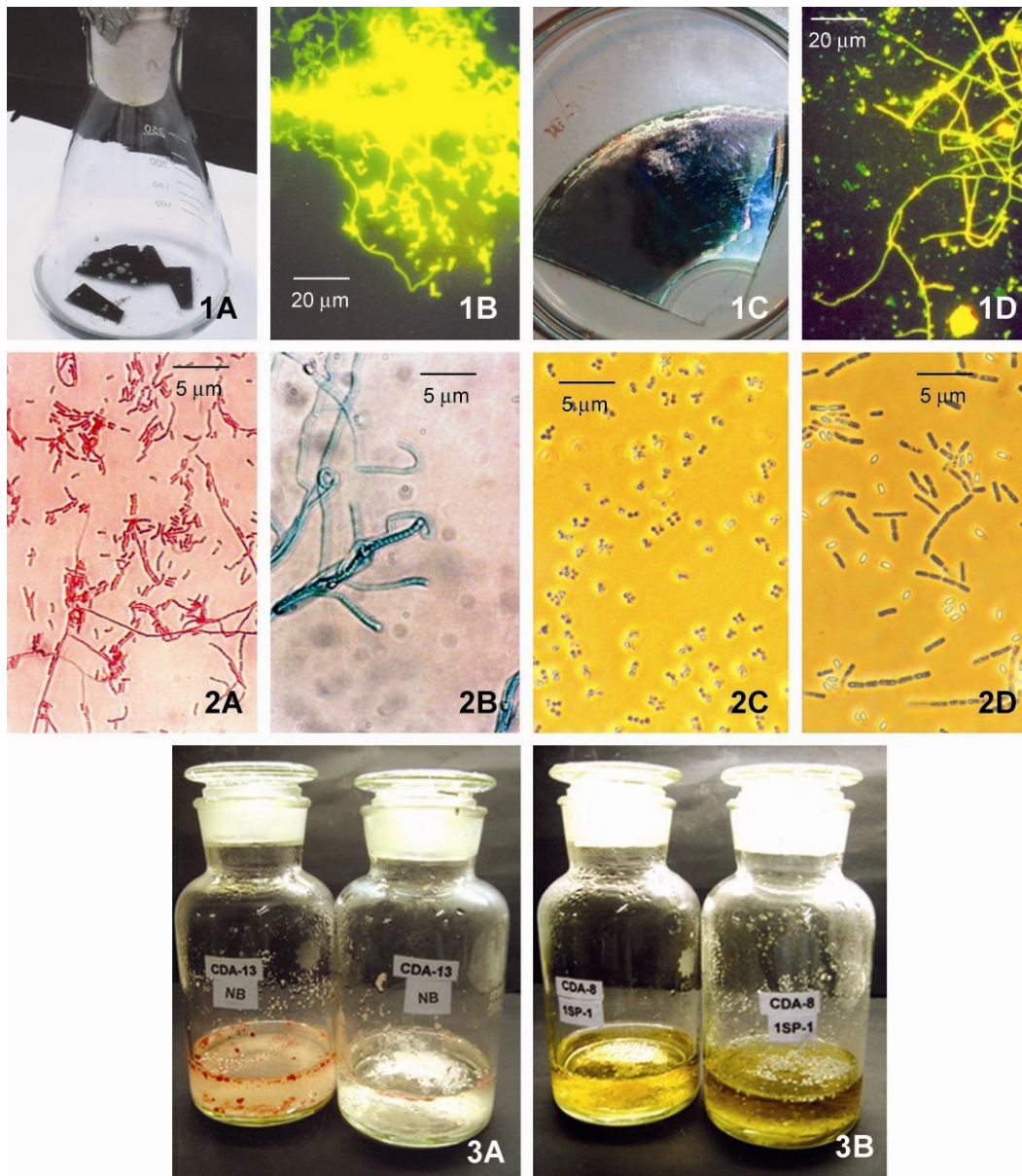


Fig. 1. Microbial growth on floppy disc (1A) and its microscopic view (1B) and on compact disc (1C) and its microscopic view (1D).

Fig. 2. Photomicrographs showing four organisms isolated from floppy and compact discs. Actinomycetes isolates are FA-3(A) *Streptomyces rimosus*, CDA-10(B) *S. halstedii* and bacterial strains are FB-1(C) *Bacillus badius*, FB-4(D) *Micrococcus sedentarius*.

Fig. 3. Utilization test of the crushed CD materials. 3A - *S. olivaceoviridis* (in ISP-1), 3B - *S. fulvissimus* (NB), CONT-control media without crushed CD material and CD - media with crushed CD materials.

The morphology of actinomycetes were studied using different methods and among these techniques whole mount under phase contrast microscopy and impression techniques were found more revealing. The isolates were filamentous, branched and coenocytic. Spiral spore chain was found in strain *S. rimosus*, *S. cyaneus*, *S. olivaceoviridis*, *S. halstedii* and *S. fulvissimus* and others produced rectiflexibles spore chain. Among all bacterial strains, *B. circulans*, *B. larvae* and *B. laterosporus* showed swollen sporangia and all showed Gram positive reaction. The biochemical and physiological tests result of twelve bacterial strains are shown in Table 1 and 15 actinomycetes strains in Table 2. Selected isolates were tested for their growth response at different pH levels (Fig. 4) and NaCl concentrations (Fig. 5).

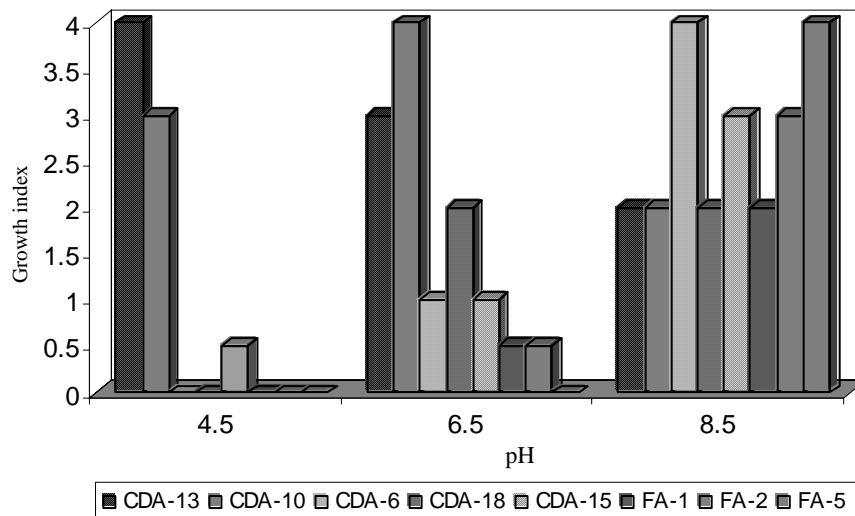


Fig. 4. Growth response of eight selected actinomycetes strains at different pH. Growth index '0' as no growth and '4' as maximum growth.

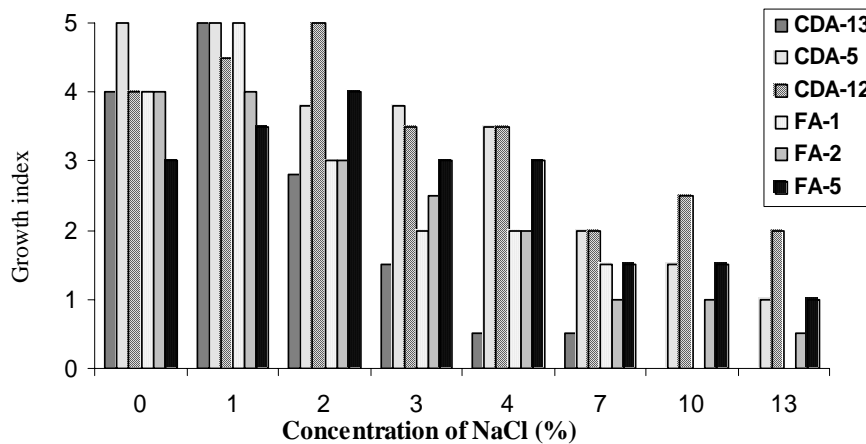


Fig. 5. Growth response of six selected actinomycetes strains at different concentration of NaCl. Growth index '0' as no growth and '5' as maximum growth.





Floppy, CD, magnetic tape and all other archiving materials are damaged in different ways and finally these are disposed off in the environment. Consequently these materials damage the ecological equilibrium of the environment.

The hydrocarbon compounds *viz.* polythene, polyvinylchloride, polycarbonate, polyester are recalcitrant in nature. It was reported that in natural conditions and laboratory conditions microorganisms like spore former bacilli caused deterioration of polythene (Khan *et al.* 2000 and Mumtaz *et al.* 2001). Biodegradation of polyester compound was also reported (Shirahama *et al.* 1996). Khan *et al.* (2000) found that on prolonged incubation, polythene strips showed perforations and disintegration. During the utilization experiment of compact discs materials, profuse growth and pigmentation were noticed in the modified media than the control (Fig. 3A-B).

Khan and Karim (1996) worked with magnetic tapes and reported microorganisms were bacteria *viz.* *Bacillus*, *Micrococcus*, *Planococcus*, *Sporolactobacillus* and actinomycetes *viz.* *Streptomyces*, *Nocardiosis*, *Intrasporangium*, *Actinomyces*. Fungal strains *viz.* *Aspergillus* and *Penicillium* were also isolated from Magnetic tape. Floppy disc is the advanced version of magnetic tape and from floppy discs and compact discs isolated microorganisms were *Bacillus*, *Micrococcus* and *Streptomyces*.

In view of the tremendous progress which is taking place in the field of electronics and technology, it may appear that floppy and CDs are the stories of a by gone days. The manufacturers have already introduced I pod, MP3 and 4, Pen drive, memory disc etc. but possibly the CDs will last for some more time in future. The floppy discs or the so called diskettes are really gone.

The work presented here indicates that in our environment, there are microorganisms which are capable of growing on the floppy discs and compact discs that results in serious deterioration in reproduction quality of these materials. This is more damaging, particularly in any humid tropical country like Bangladesh. Thus a detailed study about the nature of these organisms is required not only for the academic interest but also for undertaking any measure to safeguard our valuable archival materials.

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