

Bacterial and Fungal Testing to BS EN ISO 846: 1997

For

Construction Specialties (UK) Ltd

Final Report

Work Carried Out By

A. Smith

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Global Surface Coatings Covered



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Work Requested Bacterial and Fungal Testing to BS EN
ISO 846: 1997

Samples Submitted Replicate samples of a CS Acrovyn
2mm sheet with pebbelette grain in
Bordeaux

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I Materials Submitted For Testing

Replicate samples of a CS Acrovyn 2mm sheet with pebbelette grain in Bordeaux were received, to be tested for fungal and bacterial resistance according to BS EN ISO 846: 1997.

2 Test Procedure

2.1 Panel Preparation

The panels were dipped in an ethanol-water mixture (70:30 by mass) for 1 minute, allowed to dry at 45°C for 4 hours and then stored in sterile Petri dishes at ambient temperature prior to testing.

2.2 Fungal Resistance Testing

Spore suspensions of the following test fungi were prepared as described in the standard.

<u>Name</u>	<u>Strain</u>
<i>Aspergillus niger</i> van Tieghem	CMI 4551
<i>Penicillium funiculosum</i> Thom	CMI 114933
<i>Paecilomyces variotii</i> Bainier	CMI 40025
<i>Gliocladium virens</i> Miller <i>et al.</i>	CMI 45553
<i>Chaetomium globosum</i> Kunze: Fries	CMI 45550

Each suspension was adjusted to approx. 10^6 spores per ml and its viability checked as described in the standard before the individual suspensions were mixed in equal proportions.

The incomplete agar medium (with no carbon source) was prepared as described in the standard and poured into Petri dishes. Ten replicate panels were placed (ridged surface uppermost) individually on to plates of the solidified medium.

Five of these panels were inoculated by pipetting 0.1 ml of the prepared spore suspension over the surface of the panel and the agar (Fungal Test Batch I).

3ml of microbicidal solution were pipetted over the remaining five Petri dishes (FT Batch S). The microbicidal solution comprised a 1% solution of the agricultural microbicide benamyl, and not that given in the standard due to the non-availability of *o*-phenylphenol. (1% benamyl has been previously used in this laboratory and is known to prevent fungal and bacterial growth on surfaces).

Finally, although not required by the standard, an additional control set of 5 Petri dishes containing the incomplete agar medium only, were inoculated with the mixed spore suspension (FT Batch C).

All plates were incubated at $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 4 weeks.

2.3 Bacterial Resistance Testing

A bacterial suspension of *Pseudomonas aeruginosa*, strain NCTC 8060 was prepared and its viability checked as described in the standard.

Mineral salts agar (with no carbon source) was prepared, cooled to 45°C , inoculated with the bacterial suspension to provide approx. 5×10^4 cells per ml of agar, and poured into Petri dishes to approx. 5mm depth. Once the agar had solidified 5 replicate panels (ridged surface uppermost) were placed individually on the plates and molten inoculated agar poured over the surface to a depth of approx. 1mm (Bacterial Test Batch I).

Un-inoculated mineral-salts agar was poured into a further 5 Petri dishes and allowed to solidify. Five further panels were sterilised by dipping them in the microbicidal solution (see section 2.2) and placing them on the agar (also sterilised with the same solution). The specimens were then covered with an un-inoculated agar layer (BT Batch S).

Finally, although not required by the standard, a further 5 plates of inoculated agar were prepared as controls (BT Batch C).

All plates were incubated at $29^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and 90% relative humidity for up to 4 weeks.

3 Results and Observations

3.1 Fungal Resistance Testing

The results from the fungal resistance testing are given in Table 1, and photographic plates are included at the end of the report. Fungal growth was assessed according to the following scale:

- 0 = no growth apparent under microscope
- 1 = no growth visible to naked eye, but clearly visible under microscope
- 2 = growth visible to naked eye, covering up to 25% of test surface
- 3 = growth visible to naked eye, covering up to 50% of test surface
- 4 = considerable growth, covering more than 50% of test surface
- 5 = heavy growth covering entire test surface

After 4 weeks incubation no growth had occurred on the agar or the test sample in the inoculated plates (FT Batch I), the sterilised plates (FT Batch S) or on the agar in the control plates (FT Batch C).

3.2 Bacterial Resistance Testing

The results from the bacterial resistance testing are given in Table 2 and photographic plates are included in the report. Bacterial growth was assessed according to the following scale:

- + = growth: bacterial colonies clearly visible to naked eye
- = no growth: no colonies visible to naked eye

After 4 weeks incubation no bacterial growth had developed on the agar or the test sample in the inoculated plates (BT Batch I), the sterilised plates (BT Batch S) or on the agar in the control plates (BT Batch C).

4 Discussion and Conclusions

No growth occurred on the FT Batch C inoculated control plates demonstrating that the incomplete agar alone did not support fungal growth (due to the absence of a carbon source).

In the presence of the test material no fungal growth developed on the inoculated plates (FT Batch I), demonstrating that the test material supplied no nutritive component and hence the test fungi were unable to grow on the test sample or on the agar surrounding it.

Similarly, no growth occurred on the BT Batch C inoculated control plates demonstrating that the mineral-salt agar alone did not support bacterial growth (due to the absence of a carbon source).

In the presence of the test material (BT Batch I) no bacterial colonies developed on the inoculated agar covering the test panel, demonstrating that the test material supplied no nutritive component and hence the test bacteria were unable to grow on the test material or on the agar surrounding it.

It is concluded that over a 4-week incubation period and under the conditions of this test, the submitted material was resistant to fungal and bacterial attack.

Table 1: Fungal resistance testing

Replicate Number	Fungal Growth Assessment*					
	FT Batch I Inoculated samples		FT Batch S Sterilised samples		FT Batch C Control plates	
	By eye	X 50	By eye	X 50	By eye	X 50
(i)	0	0	0	0	0	0
(ii)	0	0	0	0	0	0
(iii)	0	0	0	0	0	0
(iv)	0	0	0	0	0	0
(v)	0	0	0	0	0	0

* Key: see section 3.1

Table 2: Bacterial resistance testing

Replicate Number	Bacterial Growth Assessment*		
	BT Batch I Inoculated samples	BT Batch S Sterilised samples	BT Batch C Control plates
(i)	-	-	-
(ii)	-	-	-
(iii)	-	-	-
(iv)	-	-	-
(v)	-	-	-

* Key: see section 3.2

PHOTOGRAPHIC PLATES

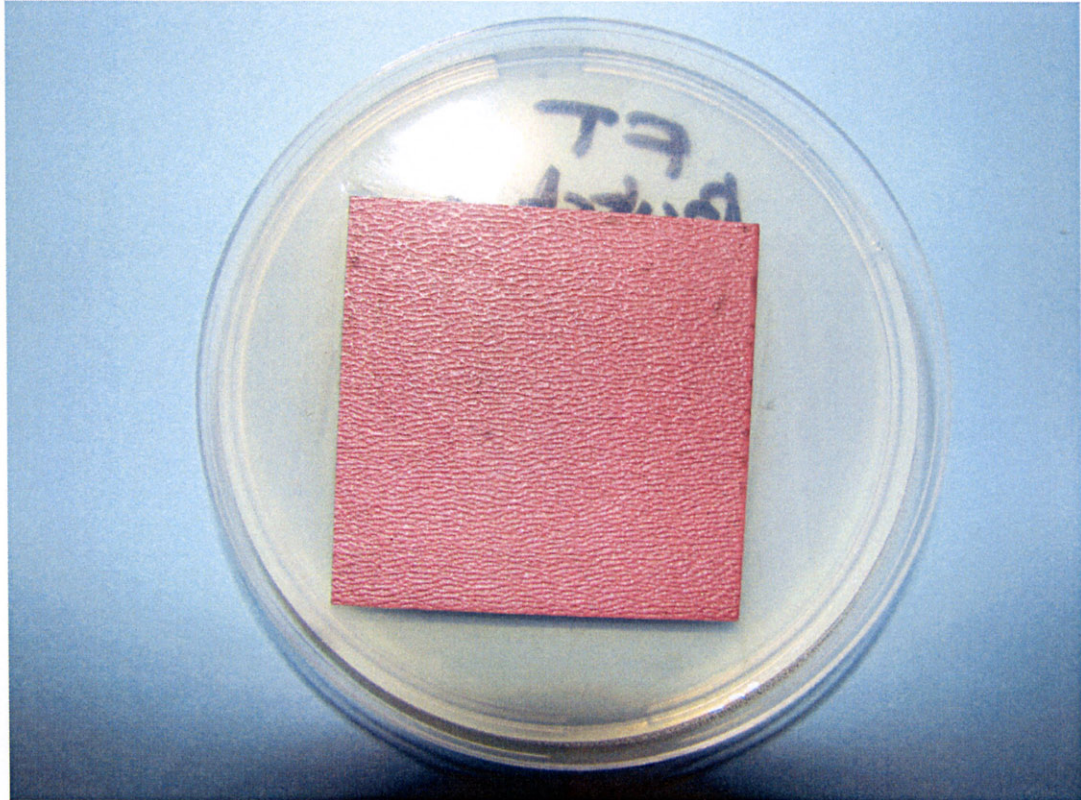


Plate 1: Fungal resistance testing - Inoculated test sample (FT Batch I) at 28 days

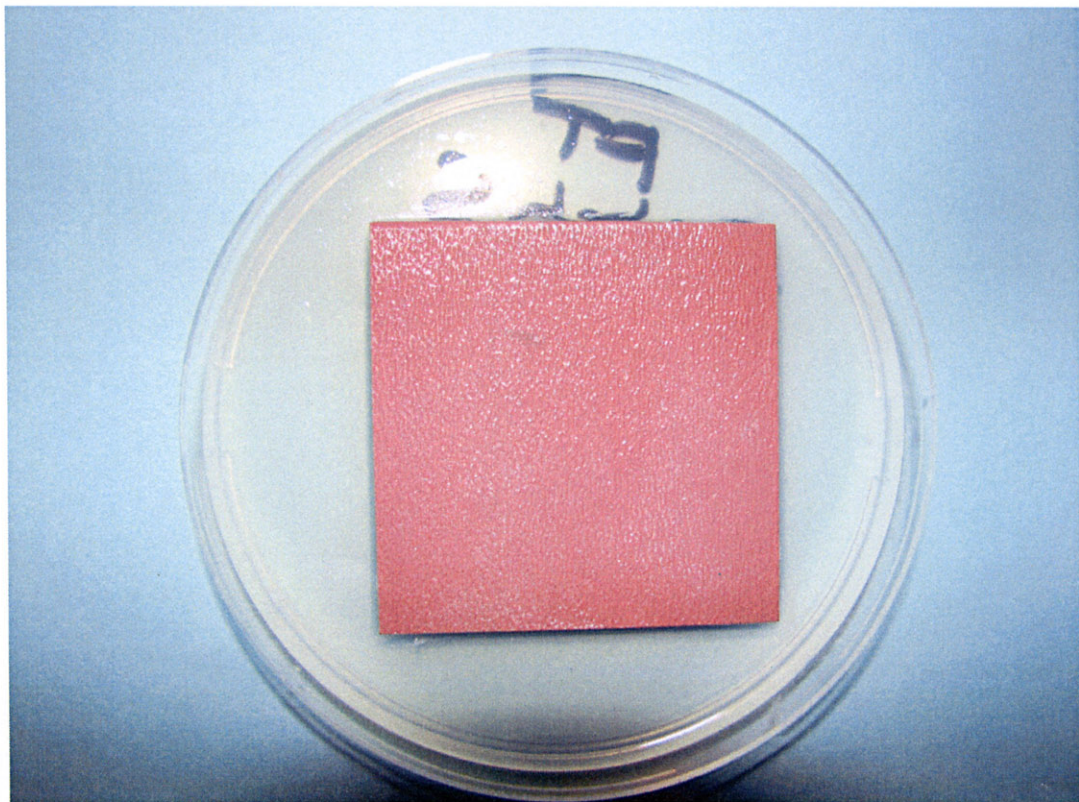


Plate 2: Fungal resistance testing – Sterilised test sample (FT Batch S) at 28 days

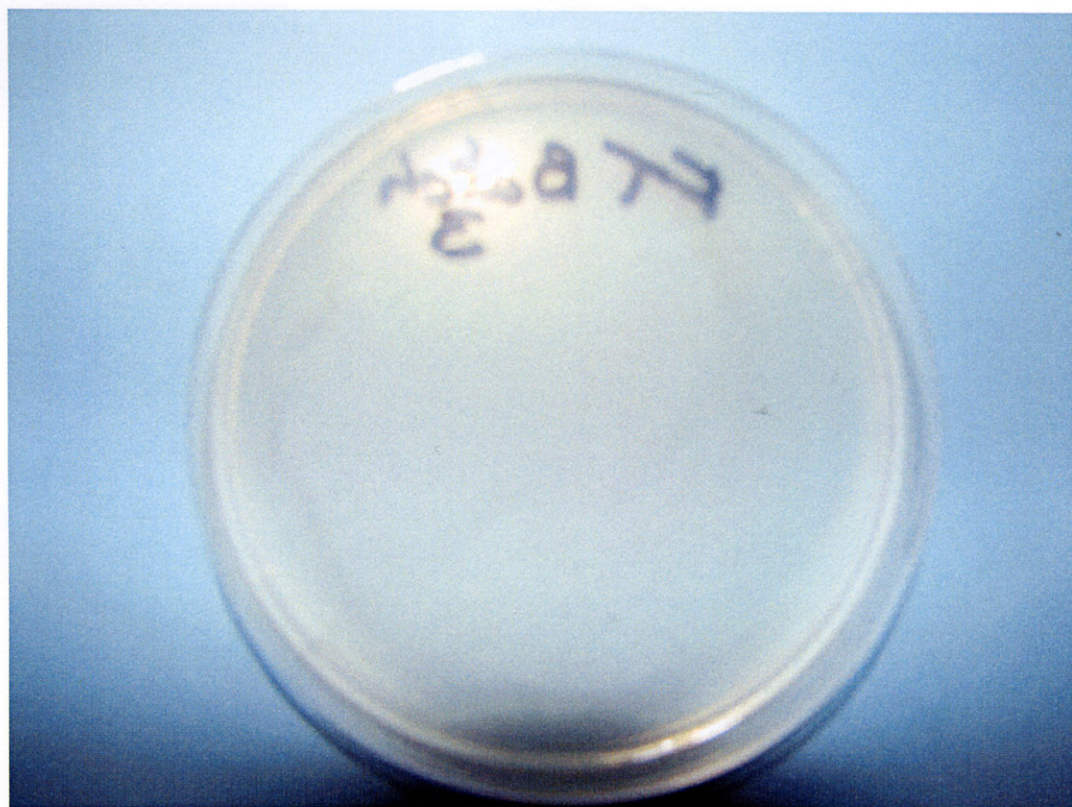


Plate 3: Fungal resistance testing – PRA inoculated control plate (FT Batch C) at 28 days



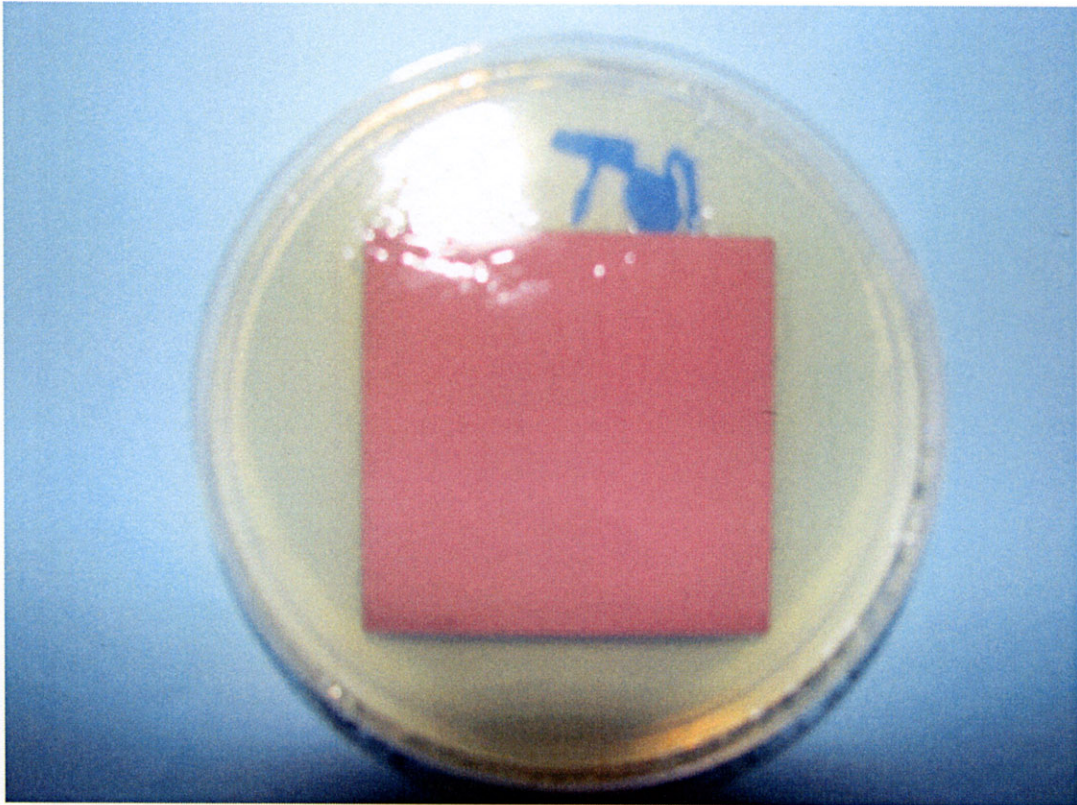


Plate 4: Bacterial resistance testing – Inoculated test sample (BT Batch I) at 28 days

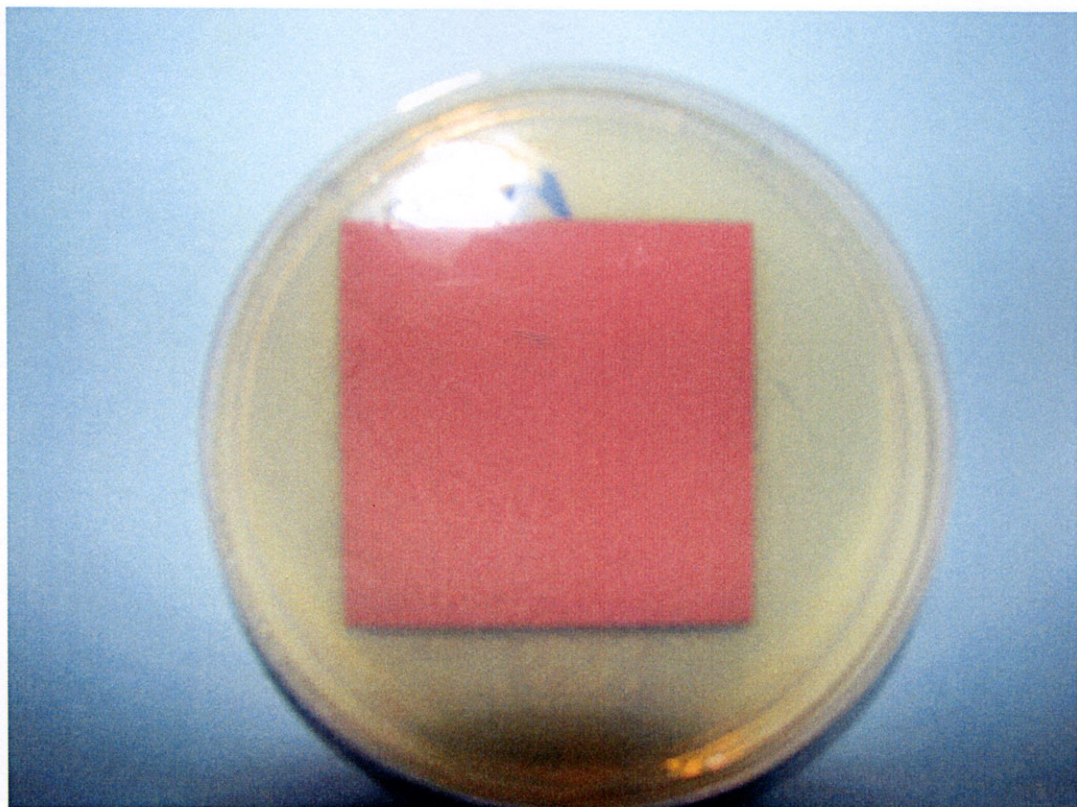


Plate 5: Bacterial resistance testing – Sterilised test sample (BT Batch S) at 28 days

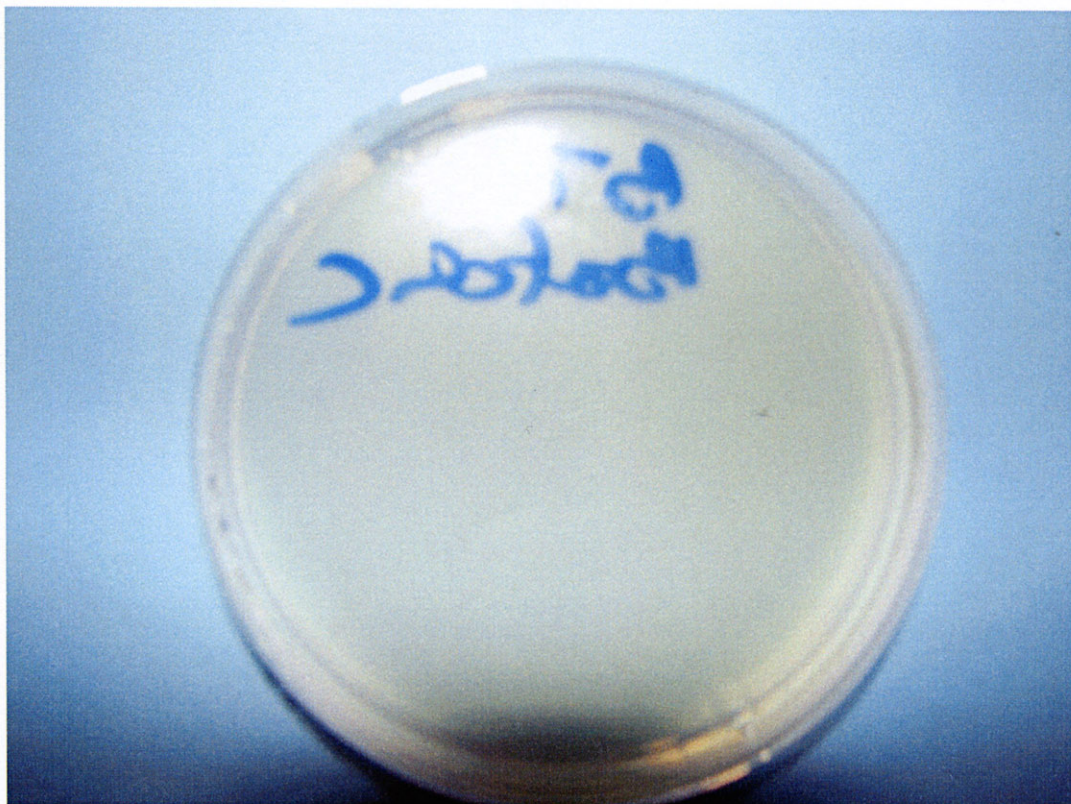


Plate 6: Bacterial resistance testing – PRA inoculated control plate (BT Batch C) at 28 days

End of Report



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