nearly 1/3 of the explored area in CIOB provides high grade regions (figure 3) for further evaluation and commercial mining.

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1. Rohrlich, V., Lin, C. and Harbaugh, J. W., *Comput. Geosci.*, 1985, **11**, 2, 215.
2. Sudhakar, M., *NIO Technical Report No. NIO/TR/9/85*, 1985, p.26.
3. Cronan, D. S. and Tooms, J. S., *Deep-Sea Res.*, 1967, **14**, 239.

## ISOLATION OF STRAINS OF *ARTHROBACTER OXYDANS* INCAPABLE OF STEROID RING DEGRADATION

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FOR accumulation of pharmaceutically important 17-ketosteroids (17-KS) in sterol fermentation, the sterol side chain has to be cleaved selectively without degradation of the steroid nucleus. The critical reaction steps in the degradation of steroid nucleus are hydroxylation at the C-9 position and dehydrogenation at the C-1,2 position[1]. When both reactions occur in the steroid nucleus, a very unstable compound, 9α-hydroxy-1, 4-androstadien-3, 17-dione, is formed resulting in spontaneous cleavage of the B ring[2]. Development of bacterial strains lacking in 9α-hydroxylase and/or 1,2-dehydrogenase is therefore of interest.

The ability of *Arthrobacter oxydans* 317, a plasmid-bearing strain for complete degradation of β-sitosterol was reported previously[3]. In the presence of hydrophobic metal chelates which inhibit 9α-hydroxylase, the strain produced 4-androstene-3, 17-dione (AD) and 1, 4-androstadien-3, 17-dione (ADD) from β-sitosterol but the production of these useful 17-KS was limited due to the lethal action of the inhibitors on the cells[4]. In this communication, the development of strains of *A. oxydans* 317 incapable of degrading AD through treatment with sodium dodecyl sulphate (SDS), a potent plasmid curing agent[5] and with 5-bromouracil (BU), a known mutagenic agent and the interesting behaviour of the new strains on the transformation of AD are reported.

*A. oxydans* 317 was grown in a number of shaken conical flasks containing 20 ml of nutrient broth (beef extract 5 g/l, peptone 5 g/l, NaCl 2.5 g/l) at 30°C. After 8 h of cultivation, SDS or BU solutions were added to separate flasks to give final concentrations of 0, 1, 4, 6, 8 and 10 mg/ml for SDS and 0, 20, 40, 60, 80 and 100 μg/ml for BU and the cultivation was continued for another 8 h. One ml of the culture broth was plated on nutrient agar plates after suitable dilution, and viable cells were counted after incubation for 24 h at 30°C. Ability of the viable cells to degrade AD was adjudged by replica plating[6] on mineral agar plates on which AD solution was spread. To study the transformation of AD, new strains refractory to AD were cultivated on a nutrient broth containing 0.5 g/l AD for 72 h at 30°C and the steroid metabolites were extracted with ethyl acetate and separated by preparative thin layer chromatography (PLC) using benzene/ethyl acetate (4:1) as the solvent phase[7]. The separated metabolites were identified from IR and NMR spectra and comparison with authentic samples.

Figure 1 shows that with increasing concentration of SDS in the treatment there was increasing appearance of strains incapable of utilizing AD until at 100 mg/ml of the chemical all the surviving cells were refractory to AD. With BU treatment a very small percentage (0.1) of the surviving cells were incapable of utilizing AD. From amongst the survivors incapable of utilizing AD, two strains (317 AI and 317 BI) from SDS treatment and four strains (ABUA1, BBUB2, ABUN1 and ABUN2) from BU treatment were isolated and transformations of AD by these strains were studied.

Strains 317AI and 317BI produced a single metabolite from AD as shown by a single metabolite spot in TLC having the same $R_f$ value as the authentic testosterone. Its identity as testosterone was confirmed by spectral data [mp: 155 C; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3520 (17-OH), 1656, 1613 ($\Delta^4$-3-C=O); NMR ($CDCl_3$) $\delta$: 0.8 (18-H), 1.8 (19 H), 3.66 (triplet, 17 OH), 5.73 (4 H)]. The results indicated the presence of active 17β-hydroxylase in the strains. Inability to produce ADD from AD which is the normal pathway of metabolism possibly indicates that the strains lack 1,2-dehydrogenase. After incu-

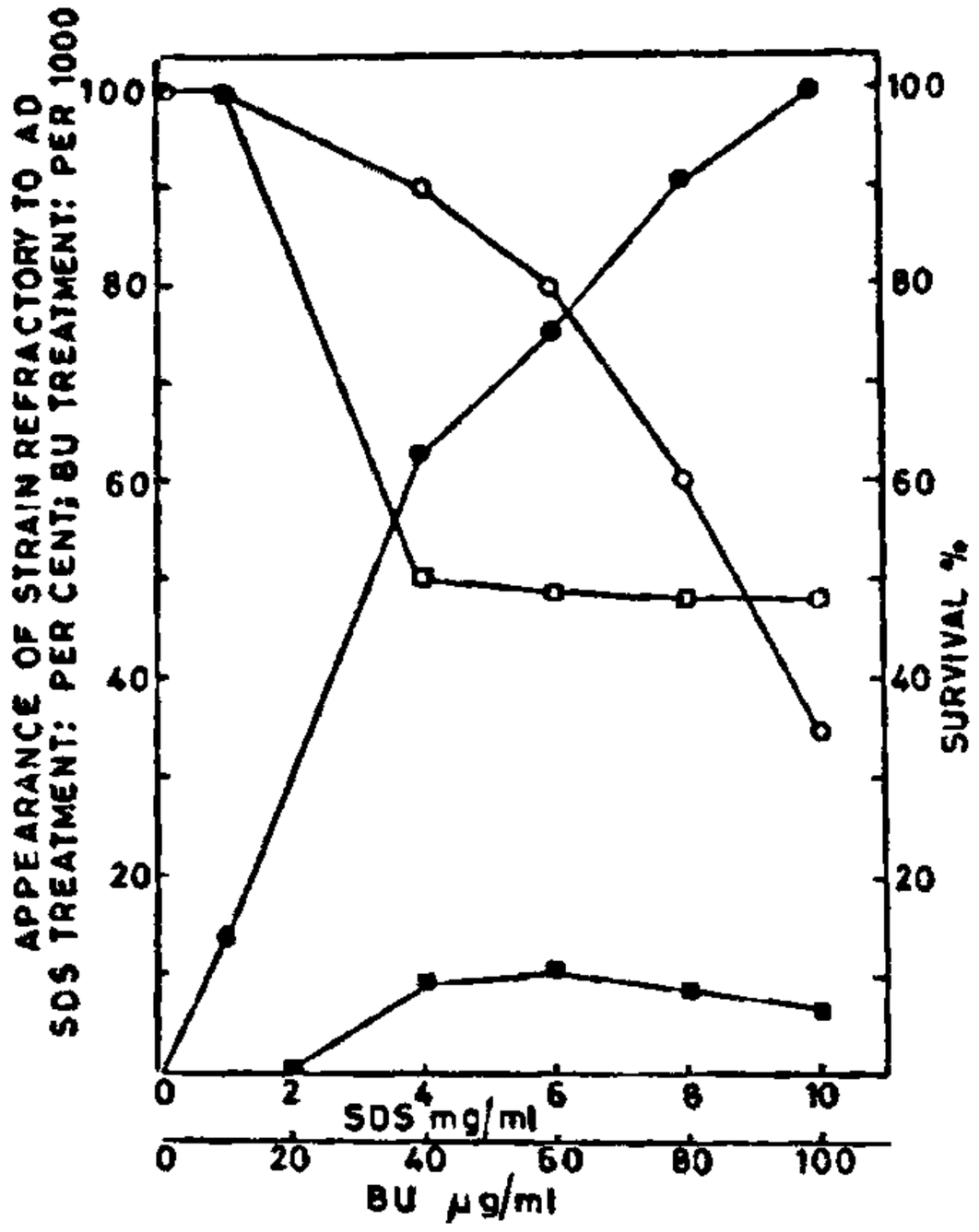


**Figure 1.** Survival and appearance of strains incapable of utilizing AD after treatment of *A. oxydans* 317 with SDS and BU. [Survival: SDS–○, BU–□; strains refractory to AD: SDS–●, BU–■.]

bation of ABUA1 and BBUB2 with AD, a strong spot corresponding to 9α-hydroxy AD and a weak spot corresponding to testosterone were obtained in TLC. The former metabolite had mp. 221–223°C, IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450(–OH), 2950, 1740(17–C=O), 1670, 1620 ($\Delta^4$–3–C=O), 1450; NMR ($CDCl_3$) $\delta$: 0.96 (18–$CH_3$), 1.26 (19–$CH_3$), 1.3–2.6(–$CH_2$–), 5.7(4–H) and it was confirmed to be 9α-hydroxy AD. No evidence for the formation of ADD was obtained indicating that the strains lacked 1,2-dehydrogenase but possessed 9α- and 17β-hydroxylases. With ABUN1 and ABUN2, the major metabolite produced from AD was ADD with testosterone as the minor metabolite and no evidence was obtained for the formation of 9α-hydroxy AD. The formation of ADD was confirmed by spectral data [mp: 169°C; IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1735(17–C=O), 1660, 1624, 1600 ($\Delta^{1,4}$–3–C=O); NMR ($CDCl_3$) $\delta$: 0.93(18–$CH_3$), 1.26 (19–$CH_3$), 6.0(4–H), 6.1 *d* (*J* = 10 Hz, 2–H), 6.93 *d* (*J* = 10 Hz, 1–H)]. The strains possessed 1,2-dehydrogenase and 17β-hydroxylase but lacked 9α-hydroxylase. The metabolite pattern observed is shown in table 1.

**Table 1** *Metabolites produced from AD by selected strains derived from A. oxydans 317*

| Strains | Treatment | Metabolites produced |
|---|---|---|
| 317 AI | SDS | Testosterone |
| 317 BI | SDS | Testosterone |
| ABUA1 | BU | 9α-Hydroxy AD (major) |
| | | Testosterone (minor) |
| BBUB2 | BU | 9α-hydroxy AD (major) |
| | | Testosterone (minor) |
| ABUN1 | BU | ADD (major) |
| | | Testosterone (minor) |
| ABUN2 | BU | ADD (major) |
| | | Testosterone (minor) |

From preliminary studies, 317AI and 317BI were found to be free from plasmid whereas plasmid was detected in ABUA1, BBUB2, ABUN1 and ABUN2. Characteristics of the first groups of strains indicate that plasmid may determine the synthesis of 1,2-dehydrogenase or 9α-hydroxylase or both. Mutagenic effect of BU may have caused the changes observed in the latter group of strains. All these strains may serve as interesting recipient or donor organisms for genetic transformation studies with plasmids for elucidating the role of plasmids in sterol transformation.

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1. Martin, C. K. A., *Adv. Appl. Microbiol*, 1977, **21**, 29.
2. Kieslich, K., *J. Basic Microbiol.*, 1985, **7**, 461.
3. Goswami, P. C., Singh, H. D., Bhagat, S. D. and Baruah, J. N., *Biotechnol. Bioeng.*, 1983, **25**, 2929.
4. Goswami, P. C., Singh, H. D. and Baruah, J. N., *Folia Microbiol.*, 1984, **29**, 209.
5. Tomeeda, M., Inuzuka, M., Kubo, N. and Nakarmura, S., *J. Bacteriol.*, 1968, **95**, 1078.
6. Hopwood, D. A., *Methods Microbiol.*, 1970, **A3**, 363.
7. Goswami, P. C., Singh, H. D., Hazarika, A. K. and Baruah, J. N., *Indian J. Biochem. Biophys.*, 1982, **19**, 367.