Buffer system employed[5].
Gel: 18.4132 g tris; 2.1014 g Citric acid; 2 l distilled water; pH 8.7
Electrode:
37.1 g boric acid; 4.8 g NaOH; 2 l distilled water; pH 8.2
Stains:
100 mg PVP; 10 mg pyroxidal-5-phosphate; 100 mg aspartic acid; 30 mg-ketoglutaric acid; 30 ml tris HCl pH 8.0
The gels were incubated at 27°C. After 10 min a mixture of the following recipes was added. 60 mg Fast Blue RR salt; 10 ml tris HCl pH 8.0

This modified staining procedure worked very satisfactory and gels with very good resolution could be reproduced in numerous cases.

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CONJUGAL TRANSFER OF STREPTOMYCIN-RESISTANCE FROM COAGULASE—NEGATIVE STAPHYLOCOCCI TO *STAPHYLOCOCCUS AURES* IN MIXED CULTURES

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TRANSDUCTION and transformation were considered to be the only means of transfer of drug resistance between strains of Staphylococci[1]. Forbes and Schaberg[2] reported conjugal transfer of R plasmids in Staphylococci by filter mating method, though they failed to transfer resistance markers in broth cultures. Fawcett *et al*[3] reported the failure of coagulase-negative Staphylococci to transfer antibiotic resistance to *Staphylococcus aureus* 1030 in mixed cultures.

Thirtyseven coagulase-negative strains of Staphylococci were isolated from 360 cases of bovine mastitis. The antibiogram of the isolates was studied both by agar diffusion[4] and agar dilution methods[5]; and ten strains were selected as donors in the study. The recipient strain used was *S. aureus* RN450 RF.

From six-hour-old cultures, 0.1 ml of the donor and 0.2 ml of the recipient were co-cultivated in 10 ml of tryptic soy broth (Difco), incubated overnight at 37° C and centrifuged at 3000 rpm for 30 minutes at 4° C. The sediment was plated on to Muller-Hinton agar (Difco) containing 2.5 mcg/ml of rifampicin and one of the antibiotics to which the donor was resistant (selective medium) and incubated at 37° C for 24 hr. (The concentrations used in the selective media were as follows:- tetracycline 12 mcg/ml; streptomycin 15 mcg/ml, erythromycin 25 mcg/ml and rifampicin 2.5 mcg/ml (Central Drugs Lab. Calcutta) and benzyl penicillin 1.0 mcg/ml (Glaxo Lab. Bombay). The donor and recipient controls were plated separately. The resistance of the transconjugants obtained was further confirmed by inoculating on to selective media containing higher concentrations of the antibiotics (rifampicin 25 mcg/ml and streptomycin 40 mcg/ml) and also by agar diffusion method.

Details of the donors, recipient, selective donor markers and transconjugants obtained are presented in table 1. All the four streptomycin-resistant donor strains were able to transfer the resistance to the

Table 1 *Details of the Donors, Recipient, Selective Donor Markers and Transconjugants.*

Coaglase-negative Staphylococci (Donor)	Selective Donor Marker	Resistance of the Trans-conjugants
M9	Str.	Str.
M22	Em., Tet.	Nil.
M37	Tet.	Nil.
M38	Str.	Str.
M41	Str., Pen., Tet.	Str.
M49	Str. Pen., Tet.	Str.
M67	Em., Tet.	Nil.
M68	Em.	Nil.
M70	Em., Tet.	Nil.
M89	Em., Tet.	Nil.

Recipient: *S. aureus* R N 450 RF phage-free, Plasmid-free Rif^r/Fus^r Abbreviations used: Str- Streptomycin; Pen- Penicillin; Tet- Tetracycline; Em- Erythromycin; Rif- Rifampin, Fus-Fusidic acid.

recipient. But the two multiple resistant strains (M41 and M49) could transfer only the streptomycin resistance and not the penicillin and tetracycline resistances. In many cases, streptomycin resistance in staphylococci have been reported to be plasmid-mediated[6].

In general, bacteriophages of *S. epidermidis* do not adsorb to *S. aureus*. Virtually all cultures of staphylococci contain high levels nucleases which can be expected to prevent transformation under natural conditions and transformation *in vitro* requires artificial conditions like 0.1 M calcium ions[1].

However, experiments were conducted to exclude the possibility of transduction and transformation. Cell-free filtrates of an overnight culture of the donors in tryptic soy broth were prepared by filtering through 450 nm and 200 nm membrane filters. Three ml of cell-free filtrate of each donor were mixed separately with 3 ml of an overnight broth culture of the recipient and incubated at 37° C for 6–8 hr and the mixture was plated on to 'selective media' and incubated at 37° C for 24–48 hr. There was no growth in any of the plates. Recipient and filtrate controls were plated separately. Failure of acquisition of resistance by the recipient organism appeared to rule out the possibility of transduction and transformation.

The studies lead to the conclusion that the mode of transfer of the streptomycin-resistant plasmid was through conjugation. Transfer of streptomycin-resistant plasmids through conjugation from coagulase-negative staphylococci of animal origin to *S. aureus* in mixed cultures has not been reported till date.

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SCREENING ROUGH LEMON AND RANGPURLIME STRAINS FOR RESISTANCE TO CITRUS SCAB

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SOUR orange scab caused by *Elsinoe fawcettii* Bitanc & Jenkins appeared in severe form in nurseries and grown up trees on Rough lemons (*Citrus jambhiri* Lush) and Rangpurlimes (*C. limenia* Osb) maintained in a compact block at the Citrus Improvement Project, Tirupati during November 1984. This facilitated locating the source of resistance, if any, in Rough lemon and Rangpurlime strains, since these two citrus varieties are commonly used rootstocks for sweet oranges and mandarins in India.

Nineteen Roughlemon and 8 Rangpurlime strains available at the project were screened under field conditions. Forty leaves and 20 fruits were randomly selected in a tree of each strain, the disease incidence was recorded using 0–4 scale and the disease index (DI) was calculated using the formula

$$DI = \frac{\text{sum of all ratings}}{\text{No. of leaves/friuts scored}} \times \frac{100}{4}$$

The strains were grouped into the following 5 categories based on DI (figures 1A & B).

Only five strains of Rough lemon *viz* Shomyndong, Milam, Khattazamir, Brazalian Rough lemon and Chase Rough lemon were free from leaf and fruit infection. The remaining fourteen Rough lemon and all the eight Rangpurlime strains showed susceptible reaction.

The seeds of immune Rough lemon strains along with one susceptible check Rangpurlime (Shrirampur) were collected and sown in pots during November 1984. The seedlings were inoculated with spore suspension after 2 months to determine their reaction to artificial inoculation. The fruits of immune strains along with susceptible check were also inoculated with spore suspension of the pathogen. Strains found immune under field conditions were unaffected, while heavy incidence of scab on leaves and fruits was observed in susceptible check.

It was earlier reported from Shahranpur that lime and lemon varieties such as sweet lime, Kagzilime, lemon Genoa, lemon seedless Tunrag, Gajanimma, limonette and sweet orange and mandarin were resistant to scab[1]. Degrees of susceptibility within the