stable until 323 K. The sharp decrease in uptake from 323 K suggests the decomposition of H_2O_2 molecule giving rise to O_2 as one of the desorbing products. It is further observed that the temperature at peak maximum (323 K, figure 1) agrees well in position if not in intensity to the desorption profile of O_2 produced from TiO_2 surface moistened with H_2O_2[8]. The formation of H_2O_2 and its decomposition on TiO_2 may proceed as follows:

$$TiO_2 + h\nu\ (\lambda \geq 365\ \text{nm}) \rightleftharpoons TiO_2 + h^+\ (\text{vb}) + e^-\ (\text{cb})$$
$$OH^-\ (\text{ads}) + h^+\ (\text{vb}) \rightarrow {}^\bullet OH\ (\text{ads})$$
$$O_2\ (\text{ads}) + e^-\ (\text{cb}) \rightarrow O_2^-\ (\text{ads})$$
$${}^\bullet OH\ (\text{ads}) + {}^\bullet OH\ (\text{ads}) \rightarrow H_2O_2 \rightarrow H_2O + 1/2\ O_2\ (g)$$

The fractional pressure dependence of oxygen photo-adsorption[8] indicates that both photo-adsorption and photo-desorption of oxygen are two simultaneous processes having opposite effects.

In conclusion, it is said that hydrogen peroxide is formed as an intermediate product during oxygen photo-adsorption on TiO_2 surfaces and is thermally decomposed at 323 K to give O_2 as one of the products.

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SPONTANEOUS MUTATION TO 0/129 RESISTANCE IN *VIBRIO PARAHAEMOLYTICUS*

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COLLIER *et al*[1] reported the vibiostatic activity in a series of 2–4 diamino 6–7 dialkyl pteridines of which 6–7 diisopropyl pteridine (0/129) had the maximum activity. Shewan *et al*[2] described the usefulness of 0/129 sensitivity test in differentiating vibrios from *Pseudomonas*. Subsequently, the importance of 0/129 susceptibility test in differentiating vibrios from other gram-negative bacilli has been emphasized by several workers[3]. Bian and Shewan[4] went to the extent of recording their belief that all 0/129 sensitive gram-negative bacilli belonged to *Vibrio* spp. However, Sundaram and Murthy[5] reported incidence of 0/129 resistance in human isolates of *V. cholerae*. In *V. parahaemolyticus* Arai *et al*[6] demonstrated 0/129 resistance associated with resistance to other antibiotics like chloramphenicol, tetracycline, streptomycin, kanamycin, ampicillin and trimethoprim. Since 0/129 sensitivity is one of the criteria for identification of *V. parahaemolyticus*, any mutation to resistance would be of taxonomic significance. Since a small percentage of the natural isolates examined in this study was resistant to 0/129, the frequency of spontaneous mutation to resistance, the stability of the resistant character and any phenotypic alterations accompanying mutation to 0/129 resistance were examined.

Single colonies of 0/129 sensitive and resistant isolates were used in the experiment. They were inoculated to 5 ml trypticase soy broth with 1% sodium chloride (TSBS) and after overnight growth were diluted and plated on trypticase soy agar containing 1% sodium chloride (TSAS) by spread plating 0.1 ml quantities. From the dilution showing isolated colonies between 30 and 300, 100 colonies were picked up and inoculated to TSBS and a young 6 hr culture was examined for sensitivity to 0/129 to study the frequency of mutation and subsequently any frequency of reversion. The representative sensitive and resistant colonies of a particular strain were examined for sensitivity to other antibiotics like streptomycin, chloramphenicol, terramycin, kanamycin, gentamycin, nitrofurantoin, gremoneg and septran. The colonies were also tested for any change in biochemical characters such as fermenta-

tion of sugars, salt tolerance, decarboxylation of amino acids and susceptibility to lytic phages. No alteration in any of these characters was observed in 0/129 resistant strains.

Out of 1787 isolates of *V. parahaemolyticus* from the natural environment, 36 were resistant to 0/129. This suggested a possibility of spontaneous mutation to 0/129 resistance occurring in the natural environment. As a first step, the minimal inhibitory concentration (MIC) of 0/129 for 6 strains of *V. parahaemolyticus* was found out. Table 1 shows the low MIC values in 4 of the 6 strains tested. In the two strains D5 and NW, the MIC was > 125 μg/ml and these were taken as 0/129 resistant strains.

The MIC of a structurally related compound trimethoprim (2–4 diamino, trimethoxy benzyl pyrimidine) was studied using the same 6 strains and the results are presented in table 1.

Results in table 2 show that in strain FCM H4 which was originally 0/129 sensitive ($0/129^s$), mutation to 0/129 resistance could be seen at a frequency of 1×10^{-6} but the frequency of reversion was too low to be detected. In the other two strains FCM NW and FCM D5 which were originally 0/129 resistant, reversion to sensitivity as well as back mutation to resistance were occurring at frequencies ranging from 1×10^{-5} to 1×10^{-6}

In strain B, the MIC of trimethoprim was < 10 μg/ml and that of 0/129 compound < 5 μg/ml. All the other strains had an MIC of trimethoprim > 300 μg/ml irrespective of their sensitivity or resistance to 0/129 thus clearly indicating that no correlation exists between the two.

Matsushita *et al*[7] demonstrated that resistance to vibriostatic agent in *V. cholerae* was borne on transferable plasmids and in several strains linked to other antibiotic markers. Even in *V. parahaemolyticus* resistance to 0/129 compound has been reported to be borne on a transferable plasmid pSA55 with a molecular weight of 11.2 megadaltons by Arai *et al*[8]. However, in this study multiple drug resistance was not observed in any of the total of 1787 isolates of *V. parahaemolyticus* examined. Even the 36 strains

Table 1 *MIC of 0/129 and trimethoprim for V. parahaemolyticus strain*

V. parahaemolyticus strains used	Source	MIC μg/ml 0/129	MIC μg/ml Trimethoprim
B	Clam	< 5	< 10
BD	Bird dropping	20	> 300
NM	Estuarine mud	10	> 300
H4	Shrimp	5	> 300
NW	Estuarine water	> 125	> 300
D5	Market drain water	> 125	> 300

showing resistance to 0/129 did not show any resistance to other antibiotics. Apart from the report of Arai *et al*[8] so far there is no documented evidence of occurrence of R plasmids in *V. parahaemolyticus*[9], though it has been reported in other related vibrios like *V. anguillarum*[10,11] and *V. cholerae*[12–16]. There are also reports of transfer of R plasmids from *E. coli* to *V. parahaemolyticus*[17] from *V. anguillarum* to *V. parahaemolyticus*[18], and from *V. anguillarum* to *V. cholerae*[19]. In view of this it is surprising that the incidence of R plasmids in *V. parahaemolyticus* strains isolated from the natural environment is so rare. It has also been noted that majority of R plasmids in vibrios are unstable[16,20,21]. Hedges *et al*[13] surmised that certain bacterial groups, notably coliform bacteria with DNA of about 50% G + C are easily able to produce a large range of R plasmid groups possibly because they were preadapted by virtue of carrying a variety of plasmids determining colicinogenicity, fermentation genes, etc on which resistant genes could be transposed. *V. cholerae* on the other hand generally contain R factors of incompatibility group C which is well known for its wide host range. Even the R plasmid detected by Arai *et al*[8] in *V. parahaemolyticus* belonged to incompatibility group C.

Prior to the work of Arai *et al*[8], only cryptic plasmids were known in *V. parahaemolyticus*. The absence of R plasmids in *V. parahaemolyticus* in this

Table 2 *Frequency of mutation and reversion to $0/129^r$ in V. parahaemolyticus*

Strain No	Character	Frequency of mutation to $0/129^r$	Frequency of reversion to sensitivity	Frequency of back mutation to to resistance
FCM D5	$0/129^r$	–	1×10^{-5}	1×10^{-6}
FCM NW	$0/129^r$	–	1×10^{-5}	1×10^{-5}
FCM H4	$0/129^s$	1×10^{-6}	Not detected	–

study as well as in those of others is perhaps due to the instability of R plasmids in this organisms. Thus this study shows that *V. parahaemolyticus* strains can undergo spontaneous mutation to 0/129 resistance.

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MICROBIAL ENZYMES IN THE PROCESSING OF OILSEEDS

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OILSEEDS and oil industry in India are of utmost importance because of their usefulness. Usually oil is extracted by the Soxhlet extraction procedure[1]. It has recently been reported that the recovery of oil from oilseeds can be increased by addition of microbial enzymes[2]. Enzymatic ability of thermophilic moulds is much superior by way of their greater thermostability and better production under nutritional conditions on a comparative basis with mesophiles[3]. Because of this higher ambient temperature (45–60°C), industrial processes are beset with less contamination and cooling costs.

The extraction procedures including Soxhlet do not allow complete recovery of oil from the seeds. The commercial recovery procedures also have similar difficulties. During the course of deterioration of oilseeds, viz. castor, sunflower, soybean and cotton by thermophilous moulds it was observed that the amount of soxhlet extractable oil increased in some cases. Since these moulds are known to secrete extracellular polysaccharases, proteases and lipases[3–5] the enzymic processing of oilseeds was studied. Available literature suggests that the mechanism of release of extra oil is based on the hydrolysis of proteins[2].

Isolates of *Aspergillus fumigatus*, *Humicola lanuginosa and Sporotrichum thermophile* were recovered from deteriorating oilseeds and maintained in YpSS medium[4]. For enzyme preparation[6], these were grown in YpSS broth in Erlenmeyer flasks for 8 days at 45°C. Culture filtrates were collected by