Results and discussion. The aflatoxin B_1 production by different fungal isolates on synthetic medium and the different varieties of soybeans is indicated in the Table. There were wide variations in aflatoxin production in different varieties of soybeans. The amount of the toxin produced was closely related to the toxin-producing potential of the fungal isolate used and the genotype of the soybean employed as natural substrate. The toxin production by the isolates of *A. flavus* was markedly lower than that compared to the production by *A. parasiticus*. The variety 'Lee' produced the lowest and the variety 'JS-2' generally resulted in the highest production of the toxin by *A. flavus* or *A. parasiticus*. The extent of difference in toxin production between less susceptible variety (Lee) and relatively more susceptible variety (JS-2) is of a higher degree in *A. flavus* series (0.125 to 1.55 or 3.125 ppm). Such a wide variation was, however, not demonstrable in the series using *A. parasiticus*.

It is generally believed that soybeans are a very poor substrate for aflatoxin production[2,3,7]. In a field study involving a survey of 866 samples of soybeans, SHOTWELL et al.[8] could observe only 0.8% incidence of aflatoxin positive, though 50% of samples showed evidence of contamination with *A. flavus*. The toxin level in the 2 positive samples was as low as 7 to 10 ppb. Again, CHONG et al.[9] failed to demonstrate the presence of aflatoxin in moldy soybeans contaminated with toxigenic isolates of *A. flavus*. However, they could demonstrate measurable amounts of toxin production using another isolate of *A. flavus* (Weybridge V. 3734/-10) which is in fact *A. parasiticus* (NRRL 2999).

Under optimal laboratory conditions, HESSELTINE et al.[2] obtained very low toxin production (0.03 to 0.08 ppm) on pearled soybeans (Hawkeye) using 3 isolates of *A. flavus*. Two of these isolates were later designated as *A. parasiticus* (NRRL 2999 and NRRL 3000). On the other hand, DAVIS and DIENER[10] obtained fairly good amounts of toxin (41 to 138 ppm) on Bragg variety of soybean after 21 days of incubation, using *A. parasiticus* (Ala-6). The results of the present series showed toxin yields ranging from 0.12 to 31.25 ppm using different varieties of soybeans infected with different isolates of *A. flavus* and *A. parasiticus*. It is interesting to note that HESSELTINE et al.[2] could get very low production (0.08 ppm) with *A. parasiticus* (NRRL 2999) using pearled soybeans (Hawkeye). It could be that this latter variety is highly resistant to toxin production, even when using one of the most virulently toxigenic isolates. The higher production of toxin in the series by DAVIS and DIENER[10] might be due to the higher toxigenic potential of the isolate used and also probably due to longer period of incubation for 21 days.

From the present series, it is obvious that soybeans do support the production of aflatoxin under optimal conditions, but the extent of toxin production is dependent on the variety of the soybeans and the toxigenic potential of the fungal isolate used. From the limited studies reported here, it is apparent that Lee variety, which supports minimal toxin production, would be suitable for extensive cultivation. This variety, nevertheless, produces appreciable quantity of the toxin when infected with *A. parasiticus*. But all available evidence appears to suggest that prevalence of *A. parasiticus* contamination is rarely encountered in India[11,12]. It is pertinent to note that the agroeconomic factors, such as yield, oil and protein contents of the Lee variety compared quite favourably with the other varieties of soybeans[13,14].

Zusammenfassung. Es wurden 5 Varietäten von *Glycine max.* mit 2 toxinerzeugenden Aspergillusstämmen beimpft und auf ihre Aflatoxinbildung untersucht. Alle Varietäten lieferten Substrate, die zur Biosynthese messbarer Aflatoxinmengen durch beide Aspergillusstämme führten.

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Improved Visualization of Wall Ultrastructure in *Saccharomyces cerevisiae*

Recently, *tris*-1 aziridinyl-phosphine oxide (TAPO) has been successfully used as a chemical fixative for biological electron microscopy[1-3]. A prefixation with a mixture of TAPO and acrolein followed by aqueous osmium postfixation produced a significant amount of new information on the ultrastructure of *Candida albicans* wall[2,4]. The results obtained in this organism cannot however be, extrapolated to the generality of yeast and yeast-like forms owing to the differences in wall chemistry and organization existing between them[5]. In particular, it was of interest to see whether the fixation procedure described for *C. albicans* could be usefully applied to *Saccharomyces cerevisiae*, a yeast 'paradigmatic' as far as wall structure is concerned.

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Methods. Two strains of *S. cerevisiae* (ATCC ns. 4098, 9696) were used throughout this study; no significant differences in wall ultrastructure were found between them. The organisms were grown in Sabouraud agar medium, 37 °C, and harvested in stationary phase of growth; then they were processed for electron microscopy essentially as described previously[4]. TAPO was obtained from Polysciences (Rydal, Penna. USA) as a 80% solution in methylene chloride (w/v), a solvent with strong extractive activity, as shown in control experiments. It was then removed by evaporation in the warm before fixing the organisms.

Results. Cells of *S. cerevisiae* fixed by TAPO-acroleinosmium (TAO) method show a dense cytoplasmic matrix, rich in ribosomes (Figure 1). The contrast of intracytoplasmic membranes is high and organelles such as mitochondria, nucleus and vacuole are well recognisable, with morphological patterns similar to that reported previously[6]. The cytoplasmic membrane is also well contrasted, sinuous, with the 'classical' trilaminar appearance and deep invaginations into the cytoplasm (Figures 2 and 3). High magnifications of the cell periphery show the distinct layering of the cell wall (Figures 2 and 4). Four layers can be seen differing in thickness, electron density and morphology of their components.

Starting from the outside, the first is a highly electron-dense layer, 45 to 55 nm thick, without a well-defined substructure; only in some sections does its outermost part show a coarsely granular aspect. The second layer, approximately 90 nm thick, is filled with a medium-density component which probably consists of intermingling fibrous glucan. In sections it appears in the form of distinct granules or granular arrays or, also, as thin fibres running in various directions and separated by narrow empty spaces.

The 3rd layer, of variable thickness, contains electron-dense material of floccular aspect embedded in an electron-light matrix. Often the dense component is seen to be continuous with a more closely packed and more electron-dense material closely pressed to the external leaflet of the plasmalemma and representing the innermost (the 4th) layer of the cell wall (Figure 4). The continuity between these two layers is more pronounced in the oldest cells.

The outstanding features of the 'bud scar' organization are shown at high magnification in Figure 3. The plug of the scar is especially rich in ultrastructural components morphologically similar to those present in the 2nd and 3rd layers of the mother wall. More laterally, an electron-lucid region is seen where, as indicated by several lines of evidence[7,8], chitin is assembled.

Discussion. Most studies on the structure of yeast cell wall have been carried out in *Saccharomyces cerevisiae* and *Candida albicans*[6]. Using permanganate as fixative for electron microscopy, the wall of these yeasts shows an almost identical ultrastructure with, essentially, a dense outer and a broad inner electron-transparent layers[6].

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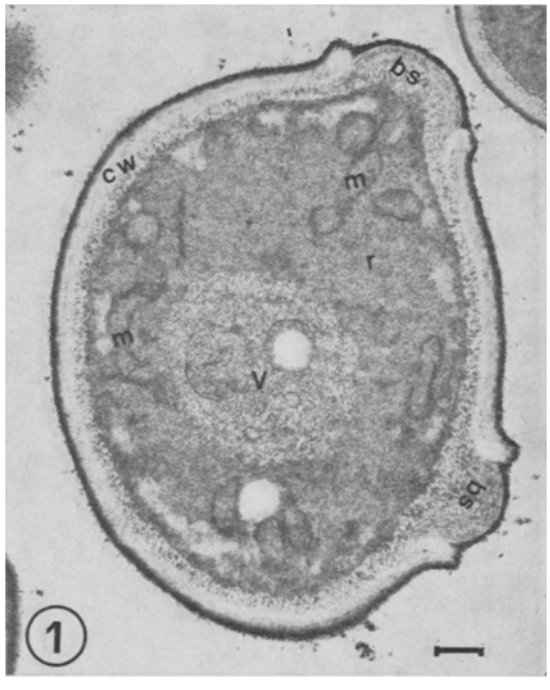

Fig. 1. An overall picture of a stationary-phase cell of *S. cerevisiae*, strain 4098. Note the good preservation of the cytoplasm and organelles. The nucleus is not shown in this Figure, but it was clearly seen in others. Two 'bud scars' are visible. Abbreviations: CW, cell wall; Cy, cytoplasm; m, mitochondria; V, vacuole; r, ribosomes; c, chitin regions; bs, bud scars. In all the electron micrographs the length of the bar corresponds to 0.2 μm.

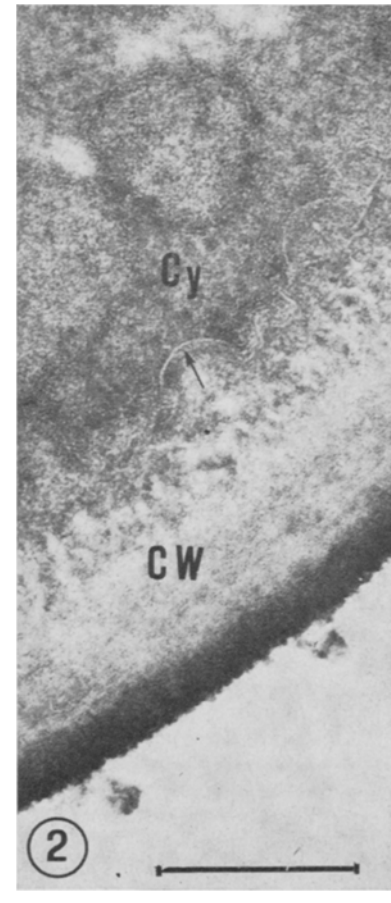

Fig. 2. High magnification of the cell periphery in the strain 4098. Note the layering of the wall. The arrow points to the plasmalemma.

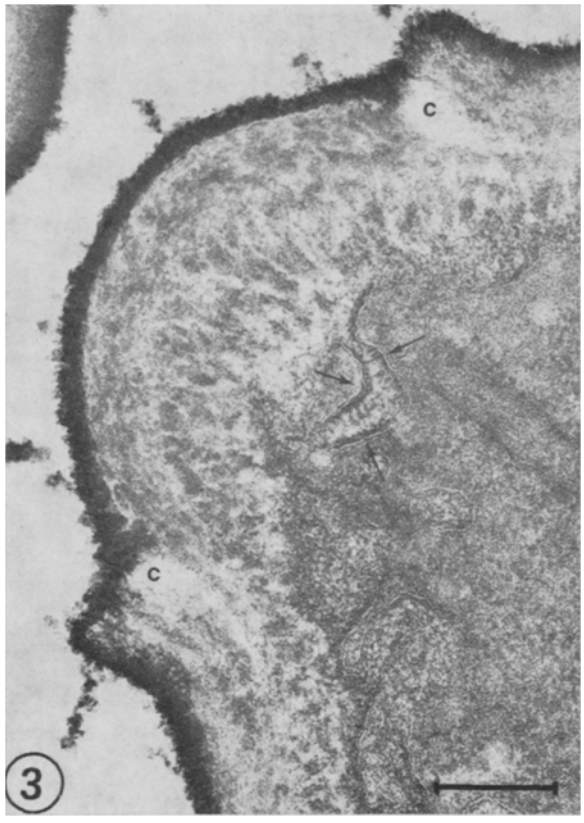

Fig. 3. A 'bud scar' in a cell of *S. cerevisiae* strain 4098. The electron-lucid annular region indicated with c is thought to consist essentially of chitin. The arrows point to the plasmalemma which circumscribes wall material.

When TAO method of fixation is used, the inner broad region of the wall is seen rich in granular and fibrous components arranged in 2 dixstinct layers (the 2nd and the 3rd described here). Furthermore, as the data reported in this and previous papers show[2,4], some significant differences in the wall architecture are found between the 2 yeasts, the main being concerned with the absence in the wall of *S. cerevisiae* of the finely fibrous outermost layer noted instead in *C. albicans* wall[2].

That cell surface could be chemically different in these yeasts was already inferred from studies on protoplasts

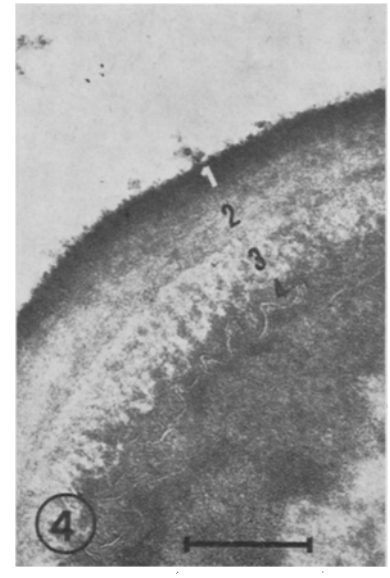

Fig. 4. Four layers of the wall in *S. cerevisiae* strain 9696 are sequentially numbered. For a description see text.

formation[9]. The different degree of preservation of the intracytoplasmic membranes in the two organisms is also indirect evidence for some difference in wall structure in view of the well established importance of the 'penetrability' of the yeast wall toward osmium fixatives in order to achieve a good preservation of internal organelles.

Riassunto. Usando fissativi contenenti TAPO é stata migliorata la visualizzazione dei componenti strutturali della parete cellulare di *Saccharomyces cerevisiae*; questa é stata paragonato con quella osservata precedentemente in *Candida albicans*.

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Detection of Encephalomyocarditis Virus Infection in Animal Cells by Gas Liquid Chromatography

The outstanding resolving power and extreme sensitivity of gas chromatography (GC) techniques has been employed for the detection of very small amounts of microbial products[1–4]. Reiner[5,6], Simmonds[7] and others used GC techniques for the identification and characterization of several microorganisms (Clostridia, Salmonellae, etc). Mitruka, Alexander and Carmichael[8,9] and Sinyak et al.[10] could detect by this technique viral infections in cells and animals.

On the assumption that viral infection brings about some specific metabolic changes in the infected cell, one would expect specific and different chromatograms from infected and uninfected cells. Such chromatograms could serve as 'fingerprints' for easy identification of a specific

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