COMPARATIVE EFFECT OF A SINGLE OR CONTINUOUS ADMINISTRATION OF «SACCHAROMYCES BOULARDII» ON THE ESTABLISHMENT OF VARIOUS STRAINS OF «CANDIDA» IN THE DIGESTIVE TRACT OF GNOTOBIOTIC MICE

R. DUCLUZEAU and M. BENSAADA

Laboratoire d'Ecologie Microbienne, INRA-CNRZ, 78350 Jouy -en-Josas (France)

_____

SUMMARY

Saccharomyces boulardii became established in the digestive tract of monoxenic mice; the number of viable cells ranged around $10^{7.5}$ per gram faeces. This yeast was drastically eliminated from the digestive tract of gnotobiotic mice harbouring a complex flora of human origin. In monoxenic mice harbouring *Saccharomyces boulardii*, *Candida albicans* became established at a level equivalent to that observed in monoxenic mice harbouring *C. albicans* alone. If gnotoxenic mice receive a concentrated suspension of viable *S. boulardii* cells so as to steadily maintain a population level close to 10^9 viable cells, *C. albicans* then became established at a level 10 to 15 times lower than that reached by the yeast strain alone. The antagonistic effect exerted in vivo by *S. boulardii* was preventive and curative. It was active against *C. albicans*, *C. krusei* and *C. pseudotropicalis* strains, but ineffective against *C. tropicalis*. This antagonistic effect disappeared when *S. boulardii* cells were killed by heating.

Key-words:

Antagonism, *Saccharomyces boulardii*, *Candida albicans*, Digestive tract, Gnotoxenic mouse.

INTRODUCTION

The ingestion of living cells of the yeast *Saccharomyces boulardii* has been known for many years. This treatment is designed to prevent or to treat digestive disorders related to disturbance of the flora of the digestive tract. It has been particularly recommended following the oral ingestion of antibiotics. Recently, a number of clinical trials have attempted to confirm the supposed effect of this treatment (2, 5, 9, 10, 15), but no experimental studies in vivo have been undertaken for this purpose. The supposedly favourable role of *S. boulardii* has sometimes been attributed to the production of vitamins (4), but much more often to an antagonistic effect against pathogenic micro-organisms which might develop in the intestinal tract of the host, in particular when the dominant saprophytic flora has been eliminated by antibiotics. This effect would be direct and comparable to that seen in vitro, in culture media (1, 3), or indirect, via stimulation of the defence mechanisms of the host (11, 12).

The prevention of intestinal infections due to *Candida albicans* is one of the effects most frequently attributed to the ingestion of *S. boulardii* (8). An antagonistic action of *S. boulardii* against *Candida albicans* has been seen in vitro (3).

The present study was designed to confirm whether this antagonistic effect seen in vitro also occurred in vivo. A simple experimental model was chosen consisting of S.P.F. animals with only *S. boulardii* and yeasts belonging to the Candida genus in their digestive tract.

MATERIAL AND METHODS

1 - Yeast strains used

The strain of *S. boulardii* No. 17 used was provided by Laboratoires Biocodex, being that used for the industrial manufacture of «Ultra-Levure» (Laboratoires Biocodex, 19 rue Barbès, 92126 Montrouge Cedex, France). The strain of *Candida albicans* No. 1677 G came from the collection of the Institut Pasteur, Paris.

The strains of *C. albicans* GR1, GR2 and GR3, *C. krusei, C. pseudotropicalis* and *C. tropicalis* were strains freshly isolated by A. Andremont from pathological material in the Department of Microbiology of the Institut Gustave Roussy, Villejuif.

2 - Culture media

All of the yeasts were cultured in the laboratory on Bx medium containing 10 g of peptone (Evans) and 10 g of gelose (Touzard and Matignon) per litre. The pH of the mixture was adjusted at pH 6.6 and it was sterilised by autoclaving at 120°C for 20 minutes. This was following by the addition of 20 g of crushed glucose (Biocodex) and autoclaving was repeated at 115°C for 20 minutes. Candida strains were cultured in liquid Bx medium or inclined Bx gel tubes. They were incubated for 3 days at 37°C with the exception of *C. tropicalis* which was incubated for 5 days. The dense suspension of *S. boulardii* used in this study were provided by Laboratoires Biocodex. The *S. boulardii* strain was cultured and filtered in accordance with the industrial process used for the manufacture of *Saccharomyces boulardii*. The paste obtained was diluted with physiological saline to give a suspension containing approximately 1×10^{10} viable cells/ml and this stock suspension was divided up under sterile conditions into 10 ml ampoules.

3 - Animals used

Axenic* mice from the C3H line and aged approximately 6 weeks at the beginning of the experiments were used. These mice were kept in plastic isolators equipped with a rapid transfer system (14) and throughout the experiment received ad libitum a standard commercial rodent diet (UAR No. 3) sterilised by radiation at 4 Mrad. For drinking, they received ad libitum tap water sterilised by autoclaving at 120°C for 20 minutes or suspension of *S. boulardii*, the viable cell contents of which are mentioned elsewhere in the text. In the latter case, feeder bottles were shaken at least twice daily in order to avoid the deposit of yeasts in the pipette.

* Axenic = S.P.F. (Specific Pathogen Free)

4 - Inoculation of the animals

Yeast inocula consisted of cultures in liquid Bx medium under the conditions described above. These cultures, which could be diffused in diluent 1 (13) in order to obtain the desired number of viable cells, were placed in sealed ampoules, introduced into the isolator and given as drinking water to animals of a given cage which had not had access to water since the previous day. One ml of dilute culture was given to each animal.

In one of the experiments, the animals were inoculated under the same conditions as above with 1 ml of 1/100 dilution prepared aerobically from the faeces of an adult human in good health.

5 - Yeast counts in animal faeces

A faecal pellet was removed directly following its emission from each of the animals of a given cage. Counts were made using a mixture of these faecal pellets. For each experiment, individual counts were made on one or two occasions for faeces from each of the animals of a given cage in order to establish possible individual variations.

Immediately following sampling, faeces were homogenised in 10 times their own weight of water, then diluted in a decimal manner. *S. boulardii* was counted by inoculation of dilutions on Bx medium with incubation for 3 days at 30°C. It should be noted that Candida strains also proliferated under these conditions.

With the exception of *C. tropicalis*, Candida strains were counted on Bx medium to which 0.5 mg/l of « Actidione » (Osi) had been added before the second sterilisation. Incubation was for 3 days at 37°C. *C. tropicalis* was counted by inoculation of dilutions on Nickerson medium (Biggy Agar, Difco) incubated for 5 days at 37°C.

RESULTS

1 - Inoculation of *S. boulardii* in axenic mice or mice carrying complex human flora

An inoculum of 4.5×10^7 *S. boulardii* was given orally to 4 axenic mice. Twenty-four hours later (Figure 1) it was found that the faecal population of *S. boulardii* in the monoxenic animals was 3 to 6×10^7 yeasts/g of faeces, remaining at this level during 60 days of observation without any notable fluctuations. On four occasions it was confirmed that populations of *S. boulardii* were identical in the 4 animals taken individually (2.5 to 4.0×10^7). Another group of 4 axenic animals received a 10^{-2} dilution of faeces from a human in good health. Eight days later, each mouse received an oral inoculum of 10^6 of *S. boulardii*. After 7 days (Figure 1) *S. boulardii* had disappeared from the 1/100 dilution of faeces from all animals.

Figure 1: Inoculation of *C. albicans* in mice harvesting *S. boulardii* and of *S. boulardii* in axenic mice or mice carrying complex human flora.
- • = total number of yeasts in gnotobiotic mice,
- o = number of *C. albicans* in dixenic mice,
- X = number of *S. boulardii* in human flora-carrying mice,
- ⌐ = threshold under which yeast strain is considered as absent.
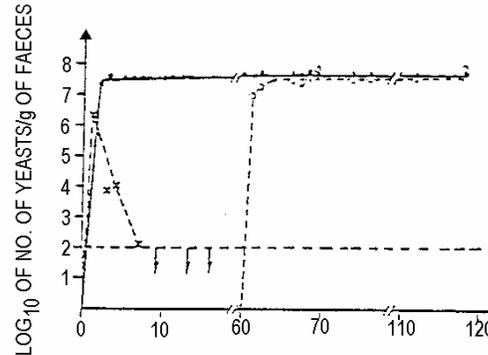
2 - Inoculation of *C. albicans* in axenic mice or in association with *S. boulardii*

Four axenic mice received oral inoculation of a culture containing 5×10^6 *C. albicans*/ml. On the following day, the mean count was $10^{7.7}$ *C. albicans*/g of faeces, and this number showed little variation during the 10 days of observation. Four monoxenic mice, in whom *S. boulardii* had been present for 60 days, received an inoculum of a culture of *C. albicans* containing 2×10^9 cells/ml. It was found (Figure 1) that 24 hours later *C. albicans* was the dominant flora in the faeces, and that during the 60 days of observation the level of this population remained stable, between 4 and 7×10^7. There was no notable variation in one animal to another (3.4 to 7.2×10^7). Using the non-selective counting medium, the total yeast count throughout this period was identical to the number of *C. albicans*. On day 70, 20 yeast colonies were taken from the dilution of 10^{-4} in non-selective medium and individually re-inoculated in the medium containing Actidione. All multiplied, and could therefore be considered as belonging to the species *C. albicans*.

3 - Effects of the continuous ingestion of *S. boulardii* on the number of *C. albicans* in the faeces of dixenic mice

A group of 4 axenic mice was inoculated with *S. boulardii* and *C. albicans*. It was found (Figure 2) that *C. albicans* established itself at a level of the order of $10^{7.5}$ as in earlier experiments. At this time, the animals' drinking water was replaced by a suspension containing 5 x 10^9 viable cells of *S. boulardii*/ml. It was then seen that the population of *S. boulardii* in the faeces of the animals became stable at a level of the order of 10^9 yeasts/g, 15 days after the administration of *Saccharomyces boulardii*. At the same time, there was a marked decrease in the population of *C. albicans*, followed by stabilisation at a level of the order of 2 x 10^6 cells/g. The same experiment was repeated in two other groups of 4 animals with very similar results (5 x 10^5 to 3 x 10^6 cells of *C. albicans*/g).
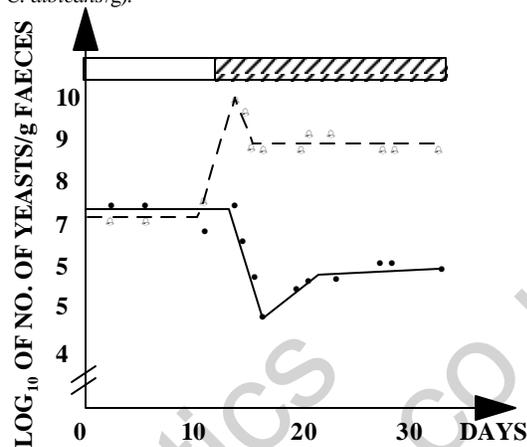
Figure 2: Effect of continuous ingestion of *S. boulardii* on the number of *C. albicans* in the faeces of dixenic mice.

• = *C. albicans*,

○ = *S. boulardii*,

☐ = ingestion of sterile water

▨ = ingestion of a suspension containing 5.10^9 cells of *S. boulardii*/ml.

In two other groups of 2 animals, the animals first received as drinking water a suspension of *S. boulardii* containing 5 x 10^9 cells/ml, then 7 days later they were inoculated with the suspension of *C. albicans*. In 24 hours the population of this strain reached 10^6 cells/g of faeces in all animals, and remained at a level of between 3 x 10^5 and 5 x 10^6 for the 21 days of the experiment.

Another group of 4 animals received a suspension containing 5 x 10^9 cells of *S. boulardii* killed by heating for 10 minutes at 100°C, this being followed by the inoculation of *C. albicans*.

During the 18 days of the experiment, the *C. albicans* count was between 3 x 10^7 and 2 x 10^8 cells/g of faeces in all animals, i.e. at a level similar to that seen in the monoxenic mice drinking plain water. The suspension of killed cells was then replaced by a suspension containing the same number of living *S. boulardii* cells. As in the previous experiments, it was found that the population of *S. boulardii* became stable at the order of 10^9/g of faeces, whilst the population of *C. albicans* fell by 10 to 50 times to became stable at between 2 x 10^8 and 5 x 10^8 cells/g of faeces.

Finally, four groups 2 axenic mice were placed in a single isolator and received respectively as drinking water a suspension containing 10^{10} viable cells of *S. boulardii*/ml and the same suspension diluted 2, 10 and 20 times. With the initial suspension, the faecal population of *S. boulardii* became stable at approximately 3 x 10^9 cells/g of faeces, whilst with the suspension diluted by half, the average count was only 1 x 10^9 cells/g of faeces.

In both of these cases, inoculated *Candida albicans* became established at a level of between 1 and 5 x 10^6 cells/g of faeces. With the other two dilutions of 1/10 and 1/20, the faecal population remained constantly below 5 x 10^8 and under these conditions *Candida albicans* became established at a level of the order of 6 x 10^7, a level reached by this yeast in monoxenic mice drinking water.
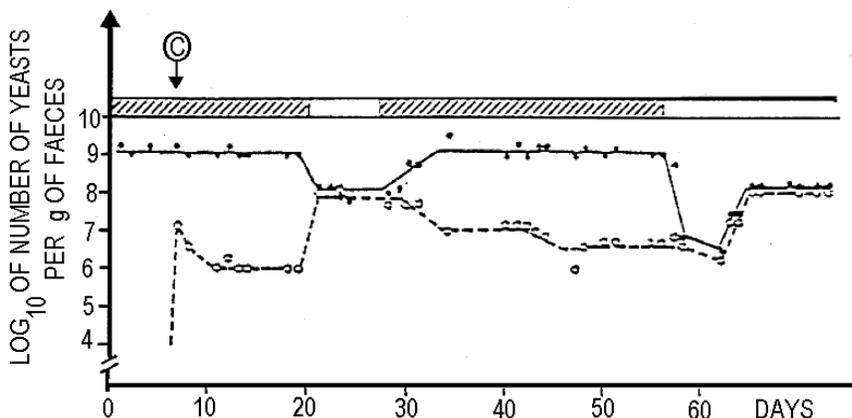
4 - Effects of the continuous ingestion of *S. boulardii* on the establishment of a mixture of 5 strains of *C. albicans*, of one strain of *C. Krusei* and of one strain of *C. pseudotropicalis* in the faeces of gnotoxenic mice

A group of 8 axenic mice received a suspension of *S. boulardii* containing 6 x 10^9 cells/ml on a permanent basis as drinking water. 8 days later, when there was a permanent count of approximately 10^9 *S. boulardii*/g of faeces, the animals were inoculated with a suspension containing 10^6 cells of 7 strains of Candida, 5 belonging to the albicans species, one the Krusei and one the pseudotropicalis species. In the medium containing Actidione, it was found that the population level of Candida became stable at approximately 10^6/g of faeces (Figure 3).

The *S. boulardii* suspension was then replaced with water. 48 hours later, the population level of Candida was approximately 10^8 cells/g of faeces. One week later, the *S. boulardii* suspension was again given to the animals. The population of Candida decreased (Figure 3) though very slowly, to become stable at a level slightly higher than that seen in the first phase of the experiment. Finally, interruption of the ingestion of *S. boulardii* was followed by a renewed increase in the Candida population to the level of 10^8/g of faeces.

Figure 3: Effect of continuous ingestion of *S. boulardii* on development of a suspension of 5 strains of *C. albicans*, 1 strain of *C. Krusei* and 1 strain of *C. pseudotropicalis* in the faeces of gnotobiotic mice.

• = total number of yeasts,
o = total number of *Candida spp.*,
= ingestion of sterile water,
= ingestion of a suspension containing 5 x 10^9 cells of *S. boulardii*/ml.
C = day of inoculation of the 7 strains of Candida.

5 - Effects of the continuous ingestion of *S. boulardii* on the establishment of *C. tropicalis* in the faeces of gnotoxenic mice

A group of 8 axenic mice receiving on a continuous basis a suspension of 5×10^9 viable cells of *S. boulardii*/ml, were inoculated with 10^6 cells of a strain of *C. tropicalis*. One day later, it was found that the population of *C. tropicalis* was at a level of 10^9 cells/g of faeces and for the next 30 days this number varied between $10^{7.5}$ and $10^{8.5}$ (Figure 4A).

Another group of 4 axenic mice receiving the same suspension of *S. boulardii*, was inoculated with the 7 strains of Candida used in the previous experiment. The Candida population became stable at a level of 10^6 cells/g faeces. The animals were then inoculated with 4×10^6 cells of *C. tropicalis*. One day later, the population of *C. tropicalis* was at a level of 10^8 cells/g of faeces, remaining at between $10^{7.5}$ and 10^8 cells/g of faeces during the subsequent 30 days of observation (Figure 4B).
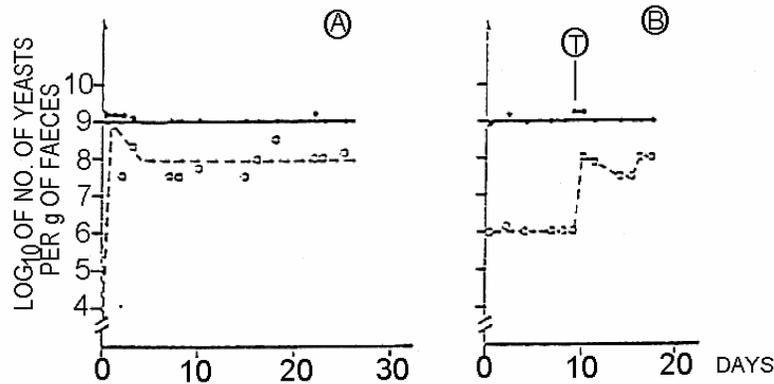

Figure 4: Effect of continuous ingestion of *S. boulardii* on the development of *C. tropicalis* in the faeces of gnotobiotic mice.

A = animals harbouring *S. boulardii* only before inoculation of *C. tropicalis*.
B = animals harbouring *S. boulardii* plus 7 Candida strains before inoculation of *C. tropicalis*.
• = *S. boulardii*,
o = total number of *Candida spp.*,
T = day of inoculation of *C. tropicalis* in group B.

DISCUSSION

The first experiment clearly showed that *S. boulardii* was capable of becoming established, i.e. of multiplying and persisting indefinitely, in the digestive tract of axenic mice. By contrast, when a complex flora of human origin had been previously transferred to the animals, the bacterial populations thus established had a drastic barrier effect against *S. boulardii*, resulting in its elimination from the digestive tract in approximately one week. In monoxenic mice, the population level of *S. boulardii* reached only 5×10^7 cells/g of faeces, whilst in monoxenic mice, with only one bacterial strain, levels were seen in general between 10^9 and 10^{10}/g of faeces (7). However, bearing in mind that the volume of a yeast is approximately 10 times greater than that of a bacterium such as *Escherichia coli*, it may be noted that the mass of microbial organic matter is similar in both cases.

Nevertheless, this mass of *S. boulardii* permanently present in the digestive tract was incapable of opposing the subsequent establishment of *C. albicans*, which rose to the same level as when the yeast was inoculated alone. Since no selective medium for *S. boulardii* was available, it was not possible to study the interaction between these two yeasts. However, it was seen that *S. boulardii* was at a minimum 20 times less abundant than *C. albicans* in the digestive tract.

Addition of a concentrated suspension of *S. boulardii* to drinking water made it possible to maintain in the faeces a yeast population 50 to 100 times greater than that established spontaneously in the digestive tract after a single inoculation. Under such conditions, there was evidence of a marked antagonistic effect against *C. albicans,* reflected by a 50 to 100 times decrease in the population of *C. albicans*. As soon as the administration of *S. boulardii* was stopped, *C. albicans* returned to its initial population level and it was found that a minimal number of 10^9 cells of *S. boulardii*/g of faeces was necessary for the antagonistic effect to be seen. This effect was preventive. The presence of high numbers of *S. boulardii* prevented the population of *C. albicans* from exceeding the level of 10^6/g of faeces. It was also curative, even though in certain cases the repression of *C. albicans*, due to the administration of a high number of *S. boulardii*, took longer to obtain and was less effective than in a preventive sense.

This antagonistic action affected not only a collection strain of *C. albicans* but also strains freshly isolated from pathological material. It was also active against other Candida species such as *C. Krusei* and *C. pseudotropicalis*. By contrast, it was ineffective against *C. tropicalis*, evidence of a certain level of specificity.

The mechanism of this antagonistic action remains unknown. It may merely be said that it disappeared after heating for 10 minutes at 100°C, indicating that it was dependent upon live cells or metabolic substance produced by them and sensitive to heat.

It is obviously very difficult to say whether the phenomenon seen here plays a role in any possible therapeutic efficacy of *S. boulardii* and, in particular, in its action against digestive pathology related to the proliferation of *C. albicans* in the digestive tract. On the basis of our result, it is evident that *S. boulardii* is incapable of establishing itself in the digestive tract in the presence of a complex flora and that, at any event, even if such establishment occurred, it would not have any effect on the population level of *C. albicans*.

Furthermore, it was shown that the effect of *S. boulardii* was seen in a mouse ingesting daily approximately 2.5×10^{10} viable cells in a digestive volume of approximately 5 ml. Estimating the digestive volume of man at 1,000 times that of the mouse, daily doses of at least 2.5×10^{13} cells of *S. boulardii* would be necessary in order to obtain the same phenomenon in man as in the mouse, whilst doses considered as high in therapeutic use consist of 6 capsules each containing approximately 10^9 viable cells.

In addition, the anti-Candida effect of *S. boulardii*, even if it occurred in man as in the gnotobiotic mouse, would not result in complete elimination of *C. albicans* from the digestive tract, but only a 50 to 100 times decrease in the population level. However, it is known that in the case of toxinogenic bacteria, a large number of live cells must be present in the digestive tract in order to cause disease (6). By analogy, it could be assumed that the antagonistic effect seen, even limited, could be sufficient to suppress the harmful effects of Candida.

It is therefore difficult to say whether the beneficial effects attributed of *S. boulardii* in human therapeutic use are due to the antagonistic effect which this yeast may have under the conditions described against *C. albicans*. However, it remains that such as an effect as the first example of a microbial antagonism phenomenon exerted in vivo by micro-organisms not forming an integral part of the «digestive tract» ecosystem, but which are capable of passing though it in high numbers without losing their viability.

BIBLIOGRAPHY

1. BIZOT, M., Phénomènes d'antagonismes entre divers micro-organismes, levures et bactéries. *Presse Méd*, 1955, 63, 1251-1252.
2. DE BOILAMBERT, P., Accidents et incidents digestifs de l'antibiothérapie. Leur prévention par les levures ultra-hautes. *Sem. Hôp. Paris (Thérap.)*, 1966, 1, 36-40.
3. BRUGIER, S. & PATTE, F., Antagonisme *in vitro* entre l'Ultra-Levure et différents germes bactériens. *Méd. Paris*, 1975, 45, 3-8.
4. BRUNEEL, M. & PATTE, F., Propriétés vitaminiques B (*in vitro* et *in vivo*) d'une levure du genre *Saccharomyces* (*S. boulardii* 17) prescrits lors de la prophylaxie et du traitement des accidents dus aux antibiotiques. *La Clinique*, 1972, 77, 197-204.
5. CASANOVA, P., Enquête praticienne sur l'efficacité de *Saccharomyces boulardii* (Ultra-Levure) dans la prévention des accidents intestinaux dus aux antibiotiques. *Méd. prat*., 1975, 591, 61-65.
6. DABARD, J., DUBOS, F., MARTINET, L. & DUCLUZEAU, R., Experimental reproduction of neonatal diarrhea in young gnotobiotic hares simultaneously associated with *Clostridium difficile* and other *Clostridium* strains. *Infect. Immun.*, 1979, 24, 7-11.
7. DUCLUZEAU, R., RAIBAUD, P., BELLIER, M. & GALINHA, A., Ensemencement de douze souches bactériennes dans le tube digestif de souris axéniques. - I. Etude cinétique de l'implantation et de l'équilibre obtenu dans les fèces des souris «gnotaxéniques». *Ann. Inst. Pasteur*, 1969, 116, 345-369.
8. GUILBAUD, J.F., Traitement des candidoses digestives et cutanéo-muqueuses par l'Ultra-Levure à haute dose. *Vie Méd.*, 1975, 8, 628-630.
9. HERVY, Ch., Essais cliniques de l'Ultra-Levure dans les diarrhées bénignes des sujets âgés. *Rev. franç. Géront.*, 1975, 6, 46-47.
10. LIGNY, G., Le traitement par l'Ultra-Levure des troubles intestinaux secondaires à l'antibio-thérapie. Etude en double aveugle et étude clinique simple. *Rev. franç. Gastroentér.* 1975, 114, 45-50.
11. MASSOT, J., DESCONCLOIS, M. & PATTE, F., Effet protecteur d'un *Saccharomyces* lors d'une infection bactérienne expérimentale chez la souris. *Bull. Soc. franç. Mycol. méd.*, 1977, 6, 45-48.
12. MASSOT, J., DESCONCLOIS, M. & PATTE, F., Effet d'un *Saccharomyces* sur les défenses immunitaires non spécifiques de l'organisme lors d'une infection bactérienne expérimentale chez la souris. *Bull. Soc. franç. Mycol. méd.*, 1977, 6, 277-280.
13. RAIBAUD, P., DICKINSON, A.B., SACQUET, E., CHARLIER, H. & MOCQUOT, G., La microflore du tube digestif du rat. - I. Techniques d'étude et milieux de culture proposés. *Ann. Inst. Pasteur*, 1966, 110, 568-590.
14. SAINT-MARTIN, B., DUCLUZEAU, R., GHNASSIA, J.C., GRISCELLI, C. & LAUVERGEON, B., Amélioration de la technologie des enceintes stériles permettant leur application à la médecine et à la chirurgie. *Rev. franç. Gynéc.*, 1975, 70, 585-593.
15. Essais cliniques contrôlés en double insu de l'Ultra-Levure lyophilisée. Etude multicentrique par 25 médecins de 388 cas. *Gaz. méd. Fr.*, 1977, 84, 1-7.