CRL1289, *Lacidophilus* CRL1266 (H_20_2-generating strains), *L gasseri* CRL1259 (organic acid producer), *L johnsonii* CRL1294 (aggregating), and *L salivarius* CRL1328 (bacteriocin producer). They have been isolated from the human vagina of women from Tucumán, Argentina, and identified by biochemical profiles, sugar fermentation patterns, and API 50 system (BioMérieux Vitec, Inc, France) [10]. NCCLS type strain, *Staphylococcus aureus* ATCC29213 from the American Type Culture Collection, was employed as reference strain.

All the microorganisms were stored in milk-yeast extract at −70°C. Prior to the assays, they were subcultured twice in LAPTg broth [16], and a third time in the media where the susceptibility to antibiotics assay was going to be performed: MRS [17], LAPTg, or Müller Hinton broth.

Antimicrobial agents

Inhibitors of the cell wall synthesis (oxacillin, aminopenicillins, ceftazidime, ceftriaxone, cefotaxime, imipenem, aztreonam, and vancomycin), protein synthesis (kanamycin, gentamicin, streptomycin, tetracyclines, chloramphenicol, clarithromycin, erythromycin, and nitrofurantoin), and nucleic acid synthesis (trimethoprim-sulfamethoxazole, rifampin; norfloxacin, ciprofloxacin, nalidixic acid, pipemidic acid, and metronidazole) were employed for inhibition tests. They were used as commercial discs (Britania, Argentina) or prepared from drugs provided by different companies (Sigma, USA; Merck, Germany; Britania, Argentina; ICN, Argentina).

Disc diffusion method

Antimicrobial susceptibility was studied by employing the method described by Bauer et al [18] for clinical isolates, modified by using three different base agar media: Müller Hinton, LAPTg, and MRS agars. Frozen microorganisms were subcultured twice in LAPTg broth and a third time in MRS, LAPTg, or Müller Hinton broth for 14 hours at 37°C. Suspensions were adjusted to tube 5 in McFarland scale (10^8 CFU/mL) and the microorganisms were (a) disseminated on the surface of MRS, LAPTg, or Müller Hinton agar plates with embedded swabs and (b) included into the agar. To include the lactobacilli into the agar, 100 μL of the microbial suspension were mixed with 12 mL of melted agar (melted and cooled down to 45°C) and then poured on plates. Antibiotic discs were placed on the surface of the agar (six discs in each plate) and the plates were incubated for 24 to 48 hours at 37°C under microaerophilic conditions. After the incubation, the diameter of the halos was measured.

Minimal inhibitory concentrations

The MICs were determined in LAPTg broth and agar. Solutions of each antibiotic at concentrations of 10 to 50 mg/mL were prepared. They were serially diluted in LAPTg broth and added to LAPTg broth or 45°C melted agar to obtain final concentrations of 1 to 1000 μg/mL. Fifty μL of exponential growth phase microorganisms at concentration of 10^7 to 10^8 CFU/mL were inoculated in LAPTg with antibiotics. Cultures were incubated up to 48 hours at 37°C and the inhibition of growth was spectrophotometrically determined at 540 mn (Gilford Spectrophotometer, USA) for assays performed in LAPTg broth and by macroscopic observation for agar tests.

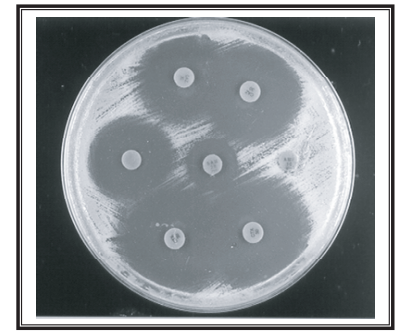

Figure 1: Semiquantitative disc assay developed in LAPTg agar for *Lactobacillus acidophilus* CRL1251 inoculated on the surface.

Statistical evaluation

The disc diffusion method was performed by duplicate and the diameters obtained for each strain are represented in the tables. MIC test was performed by triplicate. Complete inhibition of growth in all three tubes or plaques with the same antibiotic concentration was considered as the MIC.

RESULTS

Disc diffusion method

Growth of lactobacilli in Müller Hinton broth was poor and when any type of growth was detected on the agar, it was irregular and the halos were undefined. In LAPTg agar the inhibition halos were sharply defined (Figure 1) and the diameters could be easily measured when the microorganisms were inoculated either on the surface or into the agar. On the other side, the diameters of the halos for lactobacilli inoculated on the surface or into the agar were hardly different (data not shown). *L gasseri* CRL1259 and *L johnsonii* CRL1294 did not grow when they were included into the MRS agar plates while none of the six tested lactobacilli were able to grow in this media when they were spread on the surface. For those strains that were able to grow in MRS and LAPTg agars, the diameters of the inhibition halos were wider in MRS than in LAPTg agar for most of the antibiotics tested, as shown in Table 1.

In order to know whether LAPTg or MRS agar was appropriate to be used as a base medium in a standardized method for *Lactobacillus*, the effect of antibiotics on an NCCLS selected type strain inoculated in this medium was evaluated. If the halos for the type strain in LAPTg or MRS agar