Antagonism assays to identify bacterial strains producing antimicrobial compounds.

Dalia Molina-Romero[1,2¶], Antonino Baez[1], Miguel Castañeda-Lucio[3], Luis Ernesto Fuentes-Ramírez[1], María del Rocío Bustillos-Cristales[1], Osvaldo Rodríguez-Andrade[1], Yolanda Elizabeth Morales-García[1,2], Antonio Munive[1], Verónica Quintero-Hernández[4] and Jesús Muñoz-Rojas*[1].

[1]Laboratorio de Ecología Molecular Microbiana (LEMM), Centro de Investigaciones en Ciencias Microbiológicas (CICM), Instituto de Ciencias (IC), Benemérita Universidad Autónoma de Puebla (BUAP).

[2]Laboratorio de Biología Molecular y Microbiología, Facultad de Ciencias Biológicas, BUAP.

[3]Genética Molecular Microbiana, CICM, IC-BUAP.

[4]CONACYT, LEMM, CICM, IC-BUAP.

[¶]First Author

***Corresponding author**

E-mail: joymerre@yahoo.com.mx (JMR)

Abstract

Here we describe two methods for assessing the ability of one bacteria to inhibit the growth of another through the production of antimicrobial compounds. In the first assay, the double agar layer method, only the secreted substances but not the candidate antagonist bacteria interact with the sensitive microorganisms. In the second assay, the simultaneous inhibition method, both microorganisms are grown together allowing the competitive exclusion.

Culture medium for assays

Antagonism assays can be done in, any solid media, but we recommend the use of the wide-growth spectrum medium PY-Ca; opening possibilities to detect antagonisms under the same growth condition. PY-Ca contains per each liter of medium: peptone 5 g, yeast extract 3 g, Calcium chloride 0.7 g [1,2].

Antagonism assay using the double agar layer method

For this method, bacterial strains tested as possible producer of inhibitory substances are grown in liquid medium until reach the stationary phase, to do that, cultures are incubated for 24 hours (h) in PY-Ca liquid medium at 200 rpm and 30 °C. Under those growth conditions, bacterial cultures reach around 5 x 10^8 CFU/mL. Aliquots of 200 µl of bacterial suspensions are dispensed in 96 well microplates, inoculated on plates of PY-Ca with a multipoint replicator and incubated for 48 h at 30 °C. After incubation, producer colonies must be removed from the plates with a sterile glass slide and the remaining bacteria must be killed by exposure to chloroform vapor for 1 h. Plates must be left opened in a laminar flow cabinet until the residual chloroform is evaporated.

In parallel with the producer strains, each potential sensitive strain is grown at the same conditions as the potential producer strains. The culture of sensitive strain is centrifuged and the cell pellet is washed with sterile distilled water and resuspended in the same initial volume of distilled water. We recommend determine the bacterial number of this suspension, which must contain around 5 x 10^8 UFC/mL. Bacterial suspension is diluted (1:10) using water and 15 ml must be poured on solid medium PY-Ca where the producer strains were previously grown. The excess of suspension on the plates is poured in a glass beaker after 15 minutes. Plates are incubated during 48 h for visual observation of inhibition halos indicating antibacterial activity (Figure 1) [3]. In this method, microorganisms tested as sensitive ones never interact with bacterial strains tested as producers. Therefore, the halos observed are the exclusive result of inhibitory substances produced by the producer strain and that remain in the culture medium even after the cells had been removed.

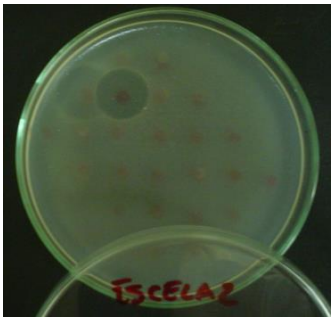

Figure 1. Antagonisms halo detected by the double agar layer method

Antagonism assay using the simultaneous inhibition method

In this method, the potential sensitive strain is grown in liquid medium PY-Ca for 24 hours. Twenty microliters of broth culture (containing around 5 x 10^7 CFU/mL) are streaked on solid agar PY-Ca plates and 20 µL of cell suspension (5 x 10^8 CFU/mL) of potential producer strain are placed at the center of the streaked plates. After 48 h of incubation, at 30 °C, the presence of inhibition halos is considered as indicative of antibacterial activity (Figure 2).

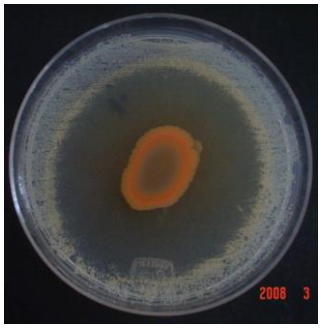

Figure 2. Inhibition halo observed by the simultaneous inhibition method

Remarks

The two methods described here could be used to explore the bacterial abilities to inhibit the growth of other microorganisms. However, inhibition results dependent on the assay, the microorganisms, and the culture media assessed.

References

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