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Au nanoparticles films used in biological sensing

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Abstract. *Lactobacillus para paracasei* are used commonly as functional food and probiotic substances. In this work Au nanoparticles self-assembled films were used for *Lactobacillus para paracasei* determination at five different concentrations. Functionalized substrates were immersed in a colloidal solution for one and a half hour at room temperature and dried at room temperature during four hours. After that, drops of *Lactobacillus para paracasei* in aqueous solution were put into the Au nanoparticles film and let dry at room temperature for another two hours. Infrared spectroscopy in attenuated total reflectance sampling mode was used to observe generation peaks due to substrate silanization, enhancement of Si-O band intensity due to the Au colloids added to silanized substrate and also to observe the enhancement of *Lactobacillus para paracasei* infrared intensity of the characteristic frequencies at 1650, 1534 and 1450 cm^{-1} due to surface enhancement infrared absorption.

1. Introduction

The development and consumption of functional probiotic foods has been increasing alongside awareness of their beneficial effects in promoting gut health as well as in disease prevention and therapy, and this has raised interest in health-promoting foods [1]. The contribution of probiotic bacteria, mainly lactobacilli and bifidobacteria, is to maintaining or improving microbial balance in the gut [1], and investigations are currently under way into their role in reducing the risk of cancer, influencing immunomodulatory features and preventing food allergies [2], counteracting hypercholesterolemia [1], and alleviating the symptoms of lactose intolerance [3]. The benefits derived from a regular intake of probiotic foods are also correlated to their ability to inhibit pathogens [4] and protect humans from gastrointestinal diseases. Nowadays, foods fortified with health-promoting probiotic bacteria are mainly produced with fresh milk or milk derivatives such as yogurt, cheese, ice cream, and desserts [5]. Functional food industries are now focusing on new non-dairy foods that can contribute to a regular assumption of probiotics in individuals with lactose intolerance or with a diet lacking milk-derived products. The suitability of cereals as a substrate for growing probiotic bacterial strains has been evaluated [6] but at present only a few functional cereal-based products are available on the market, including oat meal gruel mixed with a fruit drink containing *Lactobacillus plantarum* 299v and a rose hip drink with oats fermented by *L. plantarum* 299v [7]. To colonize the gastrointestinal tract, probiotic strains need to be ingested as large populations and on a

daily basis. Therefore, food manufacturers are trying to include probiotic strains in foods and beverages that are part of a normal diet to provide health defences while enjoying meals and to differentiate such functional products from concentrated probiotic preparations available as capsules, powders, or liquids. Research is currently under way to obtain a variety of probiotic products such as dry sausage fermented by *Lactobacillus rhamnosus* strains [8] fruit pieces containing *Bifidobacterium* spp [9], dried fruits vacuum-impregnated with *Saccharomyces cerevisiae* and *Lactobacillus casei* subsp. *rhamnosus* [10], and others products with different bacterial contents [11, 12].
On the other side, nanoparticles are widely used in the field of electronics, magnetism, catalysis, and others because of their different properties [13,14,15]. The ability of gold nanoparticles to provide a stable immobilization of biomolecules retaining their bioactivity is a major advantage for the preparation of biosensors [16], whereas in spectroscopy analysis the Surface Plasmon Resonance (SPR) effect could be used to enhance the biological signal via the SEIRA (Surface Enhancement Infrared Absorption) or SERS (Surface Enhancement Raman Scattering) effect.
In this work, the presence of *Lactobacillus para paracasei* was determined via the SEIRA effect associated to the SPR effect of Au nanoparticles films. The infrared response was observed using five different *Lactobacillus para paracasei* concentrations.

2. **Materials and Methods**

2.1 Bacterial strains and culture conditions

Lactobacillus para paracasei was obtained from Pulque, Zacatlán, Puebla. The probiotic potential of *L. para paracasei* was confirmed by adherence and antagonism tests (figure 1). Working cultures were prepared by inoculation of *L. para paracasei* strain CIBA*22* in MRS broth (Man Rogosa Sharpe, Oxoid) at 37°C during 24 h in anaerobic conditions. Working cultures were sub cultured twice before used in experiments. Bacterial suspensions were prepared, the bacterial concentration of *L. para paracasei* was determinate by optical density at 600 nm (OD600) using a MacFarland Nephelometer. For long-term storage, stock cultures were prepared mixing fresh medium (8 ml) with glycerol (2 ml, Merck) and then freezing 1-ml aliquots of this mixture at -70°C in sterile cryovials (Nalgene).
The concentrations of *L. para paracasei* used in this work were: 3 x 10^8 UFC/ml, 9 x 10^8 UFC/ml, 1.5 x 10^9 UFC/ml, 2.1 x 10^9 UFC/ml and 2.7 x 10^9 UFC/ml, **UFC** means Units Formed Colonies.

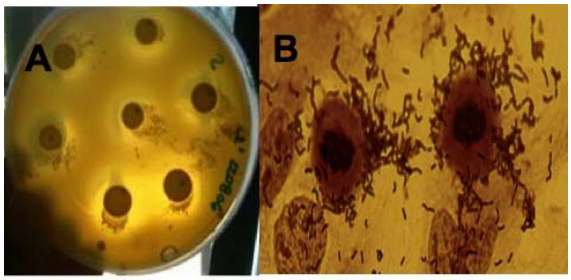


Figure 1. *Lactobacillus para paracasei.* *A.* Antagonism against pathogenic bacterial EHEC. B. Adherence on HEp-2 cells.

2.2 Colloids Solutions

Colloidal dispersions of gold (Au) nanoparticles with different sizes were prepared in a typical synthesis; solutions of $HAuCl_4$ (0.096 mmol in 25 ml of water) and poly (N-vinyl-2-pyrrolidone) (PVP, 100 mg in 20 ml of water) were prepared by dissolving the $HAuCl_4$ crystals and PVP in water. Both solutions were mixed to produce an Au (III) ion solution containing PVP as a protective polymer. Then an aqueous solution of ascorbic acid (AA, 0.096 mmol in 5 ml of water) was added to the resulting solution at room temperature. Colloidal dispersions of Au nanoparticles were formed after addition of AA solution in the mixture solution. The amount of metal ions was altered from 0.096 to 0.186 mmol in order to control the size of Au particles. Three colloids solutions were obtained with Au nanoparticle sizes: 21, 27 and 31 nm; labelled as Au-1, Au-2 and Au-3 respectively.

In figure 2 are shown an Electron Micrograph of Au nanoparticles as well as an histogram of their distribution with particle size obtained by Transmission Electron Microscopy.

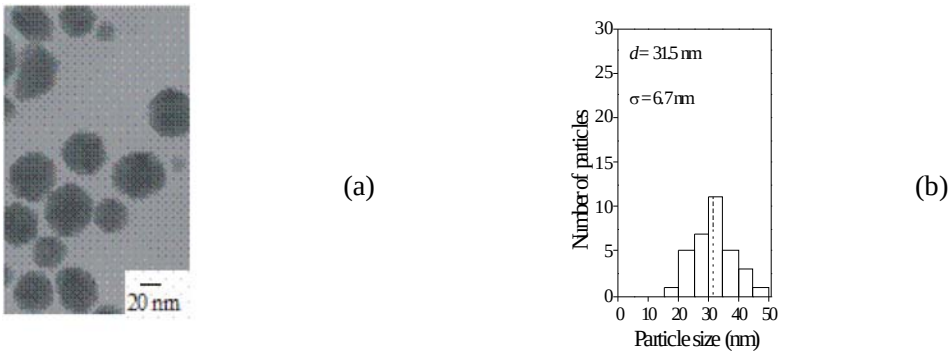


Figure 2. Electron micrograph and histogram of Au nanoparticles solution.

2.3 SEIRA Substrates

Functionalized substrates were done using the aminopropil trimetoxysilane at 1% in an acetic acid water solution. Corning glass substrates were submerged in this solution by one hour and then were cleaned using water. After that, silanized substrates were immersed in each colloid solution at two different times: a half (M) and one hour (H), at room temperature and dried by four hours at room temperature.

Deposition of Au nanoparticles was analyzed by infrared vibrational spectroscopy. The SEIRA effect on the silane molecules can be observed in figure 3, the peaks associated to silane bonds (dash line) increased their intensity due to the Au nanoparticles present in the functionalized layer (dark and grey continuous line). The peaks associated to silane have an amplification of one magnitude order; the value goes from 0.025 to 0.30 in Y axis.

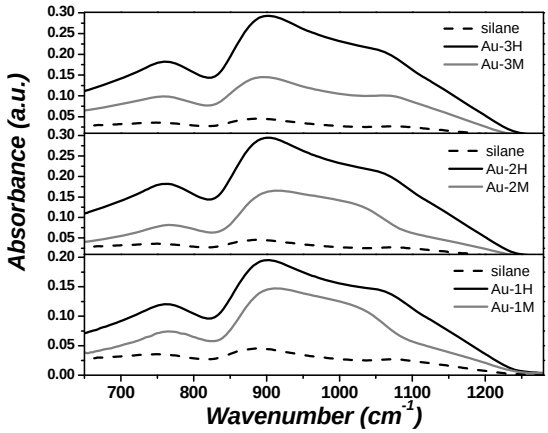


Figure 3. Infrared absorption spectra of SEIRA substrates.

Attenuated Total Reflexion sampling mode (ATR) of an Infrared spectrometer Bruker Vertex 70 was used to obtain the spectra of the figure 3. The spectrum of a glass substrate was used as background in all the measurements; the ATR crystal used was a ZnSe which has one reflection. The exposure time was 30 seconds for each sample.

3. **Results and Discussion**

Figure 4, shows the infrared absorption spectrum of *L. para paracasei* in aqueous solution form. The water baseline correction has been applied to this spectrum. The characteristic peaks associated to the bacteria are present at 2100, 1635, 1481, 1430, 1345, 1288 and 1186 cm^{-1}.

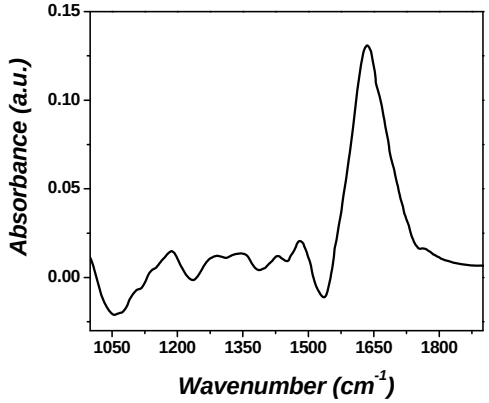
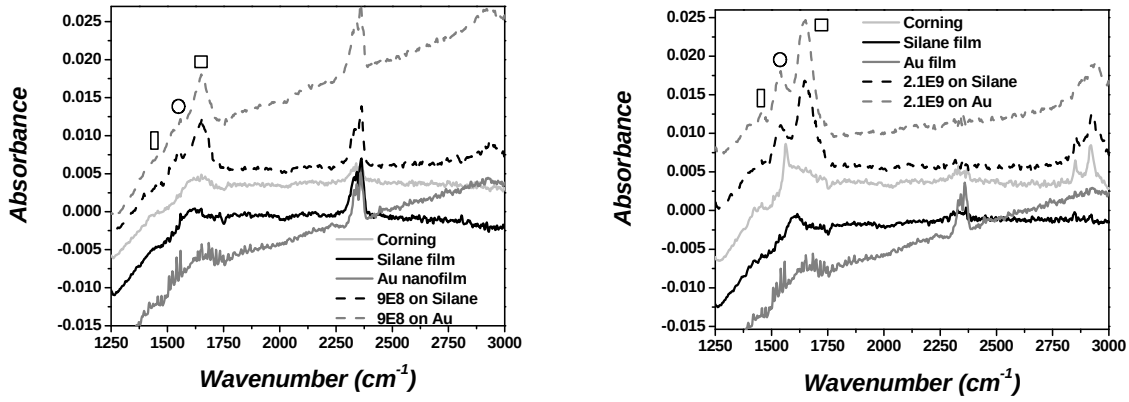


Figure 4. Infrared absorption of *L. para paracasei* infrared vibrational response in aqueous solution.

A drop of *L. para paracasei* in aqueous solution was added on the silanized substrate and on the Au nanoparticles functionalized films (glass with silane and Au). The samples were dried during two hours and then the ATR-FTIR spectra were taken.

Figures 5a and 5b show the behaviour obtained for the 9 x 10^8 UFC/ml and 2.1 x 10^9 UFC/ml, respectively. The spectra of corning (grey light continuous line), silane film (dark continuous line) and Au nanoparticles film (gray continuous line) were also added; these three spectra were translated vertically. Finally in the figure 5, dark dash line is for *L. para paracasei* on silane film and grey dash line is for *L. para paracasei* on Au nanoparticles film. The *L. para paracasei* spectra were not altered in anyway to put in evidence the SEIRA effect on the *L. para paracasei* molecules.



(a) (b)

Figure 5. Infrared spectra for the *L. para paracasei* in SEIRA substrate.

The peak at 2300 cm^{-1} is associated to the environmental ozone. In both figures, the Au nanoparticles generate undefined spectra (dark gray continuous line). The spectra of *L. para paracasei* on silane and Au (dashed lines) show peaks at 1650, 1534 and 1450 cm^{-1} (square, circle and rectangle respectively), which could be associated only to *L. para paracasei* (see figure 4). Also in the CH region (region between 2700 to 3000 cm^{-1}) is possible observe the new peaks generation at 2940 and 2852 cm^{-1}.

Table 1. Values of peak intensity for the figures 5a and 5b spectra, associated to the bacterial concentration, in silane and Au nanoparticles substrates.

Wavenumber cm^{-1}	*L. para paracasei* concentration			
	9 x 10^8 UFC/ml		2.1 x 10^9 UFC/ml	
	Silane	Au nanoparticles	Silane	Au nanoparticles
1650	0.012	0.018	0.016	0.025
1534	0.0068	0.011	0.011	0.018
1450	0.0034	0.0071	0.0064	0.013

The amplification factor observed for *L. para paracasei* is two when the SEIRA effect is present, for the 1450 cm^{-1} peak. This amplification factor is lower, when compared with a one magnitude order observed for the silane films in the figure 3; the experimental values are shown on table 1. This behaviour could be due to the lower concentration of the bacterial on the film.

4. Conclusions

We have proved the use of silane and Au nanoparticles functionalized films, deposited on glass substrates to observe the enhancement of the infrared absorption intensity of the biological element *Lactobacillus para paracasei.* The mean peaks observed for this material, located at 1650, 1534 and 1450 cm^{-1} where enhancement in intensity which could be due to the SEIRA effect.

5. References

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