

Original Research Article

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## Detection of *Bacillus* sp. from Wall Paint

Eman Aboob Mukhaifi, Wijedan H. Al-Tamimi and Ahmed A. Burghal

Department of Biology, College of Science and University of Basrah, Iraq

\*Corresponding author

### ABSTRACT

#### Keywords

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Bacteria which grow on the surface of such paintings might discolor the painting not only through their own pigments but also by excreting metabolic products. Heterotrophic bacteria can use organic compounds from the paint layer as growth substrates, producing acids, which cause discoloration of the paint or change its consistency. The bacterial contaminants isolated in the paint products included *Bacillus* sp. All isolates can grow in MSM medium with paint and cause some changes color, turbidity, acidity, decrease amount of paint and high to moderate growth of *Bacillus* sp.

### Introduction

Paints are uniformly dispersed mixtures having a viscosity ranging from a thin liquid to a semisolid paste, consisting of a pigment suspended in a liquid vehicle such as oil or water. With a brush or roller or spray gun, paint is applied in a thin coat to various surfaces such as wood, metal, or stone. Although, their primary purpose is to protect the surface to which they are applied from corrosion, oxidation, environmental weathering or other types of deterioration, paints also provide decorative finish (1). The components of paints include vehicle, pigment, additive and solvent. The various organic materials of paints represent a carbon source for practically all species of microorganisms and act as nutrients to

stimulate microbial growth both in-can and on the dry paint film. This seriously compromises the adhesion and durability of the paint as well as its decorative function (2). Unfortunately, microorganisms contamination of paints can be from a number of sources such as raw materials, manufacturing plant process units and packaging materials the major groups of microbial involved in paint deterioration are bacteria and fungi, which can grow on applied paint films and solvent and water based coatings (3). Most commonly isolated bacterial species in paints include *Enterobacter*, *Proteus*, *Escherichia*, *Bacillus*, *Pseudomonas*, *Micrococcus*, *Serratia*, *Aeromonas* and range of anaerobic bacteria including *Bacteroides*, *Clostridium*, *Desulphovibrio* and *Bifidobacterium* have also been isolated in paints. Much information is

given on paint and coatings, physical and chemical nature of paints structure formation, the results of investigations of physical and mechanical values, however, the data on complex investigations of the surfaces already finished are insufficient. Usually, mechanical and physical values of individual components, the coating and the wall being painted are known (4). For the wall to be painted, optimum selection of paint is necessary. In case of bi-laminar system paint film, the wall being painted two opposite processes take place, water flow rate from outside towards the wall, and water vapor flow rate of the wall to outside. Water vapor accumulated in the wall, when disturbed from escaping through a very dense film might cause blebs, or tear off the whole film or its parts. It was foreseen before investigations of paints on durability that theoretical attitude low vapor (1). Bacteria participate in the mineralization of paints through biofilm formations on the surfaces like stone buildings causing aesthetic and structural damage. Various types of organisms are involved in paint spoilage and they include bacteria, fungi, algae, and protozoa. The interactions between these organisms can enhance or retard the overall rate of paint biodegradation (5).

## **Materials and Methods**

### **Samples collection**

The paint samples were collected from various shops of the city of Basra and from different factories and at different prices

### **Isolation of *Bacillus* sp. from wall painting**

10 ml from a liquid paint sample were transferred aseptically to 90ml distilled water into 100 ml flask and then put in a rotating shaker with a speed of 150 rpm at 30°C for 30 min from which 1ml transported from solution was pipetted to 9ml distilled water the purpose

was to make  $10^{-1}$  decimal and provide inocula of the dilutions ( $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$ ) by sterilized pipette into nutrient agar of Spread plating, respectively in three replicates. Incubation was carried out at 37°C for 24 h. (8).

### **Isolate maintenance**

Isolates were maintained in nutrient agar screw capped- tubes covered with 20% glycerol (6).

### **The pH of wall painting**

The ratio of paint: water suspension was used as 1:1 for pH measurement by pH-meter after testing by standard solution (6).

### **Determination of behavior of *Bacillus* sp. in MSM medium**

*Bacillus* isolates were inoculated into Mineral salt medium MSM consist of NaNO<sub>3</sub> 2.5g, K<sub>2</sub>HPO<sub>4</sub> 1.0g, KH<sub>2</sub>PO<sub>4</sub> 0.5g, MgSO<sub>4</sub> 0.5g, KCl 0.1g, FeSO<sub>4</sub> 0.01g CaCl<sub>2</sub> 0.01g, NH<sub>4</sub>NO<sub>3</sub> 0.39, Na<sub>2</sub>HPO<sub>4</sub> 5.67g, Glucose 30g and 1000ml of distilled water, supplemented with wall painting. The cultures were incubated at 37°C for 30 days and agitated at 150 rpm. The growth was measured in term of OD at 600nm by spectrophotometer. Each sample was done in triplicate. Turbidity of bacterial solution after incubation compared with the standard test tube McFarland for 108 cells / ml of stuck bacterial. Color and amount of wall painting noticed through the vision of the eyes (1).

## **Results and Discussion**

Isolates of *Bacillus* sp. were (7) isolates from painting wall. All isolates can grow on nutrient agar. From the morphological examination, all isolates were gram positive, short chain bacilli and spore forming. Cultured *Bacillus* species test positive for the enzyme catalase if oxygen has been used or is present.

Are shown in (Figure 1 and 2). High significant difference was observed of behavior the *Bacillus sp.* In liquid medium with wall painting after 30 days incubation in shaking incubator with an LSD = 0.0801 with  $p < 0.01$  table 1 and figure 3.

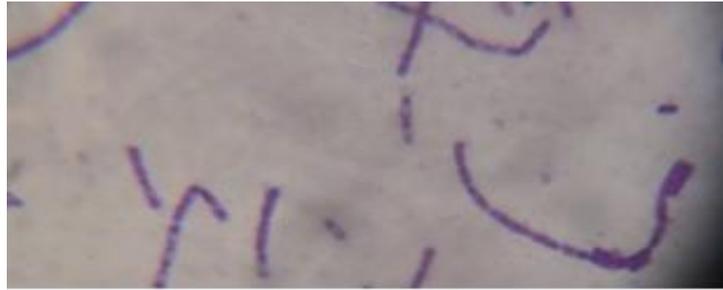
**Table.1** Different behavior of *Bacillus sp.* in MSM medium after 30 days

| Isolates | Appearances |           |                 |              |                 |
|----------|-------------|-----------|-----------------|--------------|-----------------|
|          | Growing     | Turbidity | Color of medium | pH of medium | Amount of paint |
| Control  | uncultured  | Absent    | white           | neutral      | high            |
| A        | moderate    | moderate  | yellow          | acidity      | low             |
| B        | high        | Dense     | yellow          | acidity      | low             |
| C        | moderate    | moderate  | yellow          | acidity      | low             |
| D        | moderate    | moderate  | yellow          | acidity      | low             |
| E        | high        | dense     | yellow          | acidity      | low             |
| F        | abundant    | low       | yellow          | acidity      | low             |
| G        | abundant    | low       | yellow          | acidity      | low             |

**Fig.1** *Bacillus sp.*



**Fig.2** Gram stain of *Bacillus sp.* magnification 1000X



**Fig.3** Behavior of *Bacillus sp.* in MSM medium with wall painting



From results in shape (1) the Spread plating is the way appropriate to isolate the bacteria contaminated the paint. There are a number of procedures available for the isolation of microorganisms from mixed culture. But the initial and the most simpler method of isolation is spread plating on solid agar medium. The purpose of spreading is to isolate individual bacteria (7, 9). The majority of *Bacillus sp.* that have been found in wall paint, due to the raw material in paint such as pigments, solvents, resins, and various additives. The pigments give the paint color solvents make it easier to apply resins help it dry. Hundreds of different pigments, both natural and synthetic, exist. The basic white pigment is titanium dioxide, selected for its excellent concealing properties,

and black pigment is commonly made from carbon black. Other pigments used to make paint include iron oxide and cadmium sulfide for reds, metallic salts for yellows and oranges, and iron blue and chrome yellows for blues and greens (11, 10).

Result in table 1 and shape (3) show different behavior of *Bacillus sp.* in MSM medium compared to (9). Because of Paintings, whether easel or mural, include a wide range of organic and inorganic constituents and provide different ecological niches that may be exploited by a large variety of microbial species. Many of the components of paintings are biodegradable, and so are the additives glues, emulsifiers, thickeners that facilitate drawing or application

of paint layers or enhance the aesthetic quality of the finished product (12). Increasingly, it has become evident that wall paints can be degraded by the presence and or activities of microorganisms bacteria can reduce the shelf-life and degrade the quality of paints.

The effects of *Bacillus sp.* that can grows in paint irrespective of the additive added depends on many facto of which storage is of prime important, can oxidize organic matter using electron acceptors an oxidation-reduction types of reaction that leaves large acidic fragments in the paint than oxygen for nutrients, during their metabolic process plays its spoilage role differently from their aerophilics counterparts.

The associated spoilage is indicator of loss viscosity of paint. pH and color of medium changes as a results of bacteria metabolites liberated to the paints, frothing, sedimentation and separation into phases, discoloration, aesthetic degradation and potential health hazard and contaminated of paint (13).

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