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Airborne Microorganisms Present in the Atmosphere in Valladolid, Spain

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Abstract

Over the past few years, research on the quality of air and microorganisms present in the atmosphere and spore composition of the environment has increased significantly, due to concerns over health risks for humans, plants, and animals. This study shows the abundance and diversity of microorganisms and the atmosphere of an urban nucleus, that is, the city of Valladolid (Spain). We considered the conditions of precipitation, humidity and wind, and the presence of some atmospheric pollutants such as nitrogen dioxide and nitrogen monoxide (NO_2/NO), carbon monoxide (CO), sulfur dioxide, and particulate matter (*PM*10 and *PM*2.5). After their deposition present at five geographic points with different environmental conditions, differences were observed in the proportion of bacteria growth which was characterized by growth in several specific culture media. Most identified the Gram-negative bacteria identified in the air samples collected belong to the genera *Staphylococcus* and *Streptococcus*. Gram-positive bacteria were present at a low rate.

Keywords

Air Quality, Environmental Conditions, Atmosphere Pollutants, Valladolid, Airborne Microorganisms

1. Introduction

In recent years, interest in the quality of atmosphere that we breathe is increasing. Although the atmosphere does not have specific autochthonous microbiota, it is a medium for the dispersal of different microorganisms (spores, bacteria, viruses, and fungi) from other environments. It is estimated that under normal conditions there are between 100 and 1000 microorganisms per liter of air, suspended in dust and sand particles carried by the wind [1] which represent hundreds of unique taxa [2]. However, the diversity, distribution and interactions of these microorganisms are poorly understood.

Airborne microorganisms are of great importance when interacting with terrestrial life forms because they can be a source of pathogens able to cause diseases in plants, animals, and humans [3]. Among diseases caused in humans, respiratory and allergic ones must be highlighted [4].

There is currently great interest in the microbiology of the atmosphere, since it is linked to epidemiological and dispersal aspects and biogeography, but also in determining the activity, adaptations, and functions that the resident microbial populations develop in this specific habitat or ecosystem. Among these functions we highlight bio-geochemical transformations of the nitrogen cycle (mineralization of organic nitrogen and nitrification) in clouds, and of simple organic compounds [5]. Thanks to new remote sensing techniques, air-quality monitoring programs and improvements in the detection and characterization of microorganisms and the dispersal patterns of microorganisms can be designed [1].

Residence times of the microorganisms in the air may vary depending on the size of the particles with which they are associated, together with the air temperature and relative humidity, among other factors. Although the studies reviewed to date indicate that microorganisms vary in abundance, distribution and diversity in the atmosphere, the patterns in the variation of microorganisms in the atmosphere have not yet been well documented [6]. Since the interaction of biological particles in atmospheric processes is directly related to the surfaces of the particles together with the processes mediated by their active metabolism. Microorganisms and other types of biological particles have properties that enable them to act as cloud condensation nuclei and thereby contribute to cloud formation. Some of them also produce very active ice cores that may be involved in processes of precipitation [7].

Approximately 25% of airborne particles in the size range 0.2 to 50 µm are primary particles of biological sources such as animals, humans, and vegetation [8]. Likewise, the concentrations and composition of airborne microorganisms vary depending on environmental conditions, which have daily, weekly, and seasonal changes [9] [10]. Microbial contamination causes allergies, infections, and toxicity, and in the case of urban areas, microbial concentrations in the urban environment are influenced by human activities [11] [12]. Airborne microorganisms are metabolically active, and they can maintain their viability in atmosphere. They may change the chemical components of aerosols in suspension, thereby modifying the chemistry of the atmosphere [13] [14]. Additionally, an increase in microbial concentrations in the air has been observed with increasing atmospheric CO_2 concentrations [15].

A study has shown that most bacteria detected in the atmosphere are Gram-positive and include spore-formers such as *Bacillus* and *Microbacterium spp.* Gram-negative bacteria are also present in the atmosphere despite occasional extremely adverse conditions. In addition, the presence of fungi, spores of *Cladosporium*, *Aspergillaceae*, *Alternaria*, *Botrytis* and various *Basidomycetes* have been frequently observed in the atmosphere, but spores of *Cladosporium spp*. seem to predominate [9].

The purpose of this study was to identify the genera of bacteria found in the atmosphere and to determine the existence of an interrelationship between the number of viable bacteria and atmosphere pollutants, as well as between the number of viable microorganisms and weather, and to correlate this information with atmosphere pollution and weather parameters.

2. Materials and Methods

2.1. Study Area

Valladolid is a city of Spain located in the northwest of the Iberian Peninsula, capital of the province of Valladolid, located in the center of the northern plateau of Spain (Longitude: O4˚43'25.39" Latitude: N41˚39'18.65") with a total area of 197.91 km^2 and a population of approximately 300,000. This plateau, surrounded by mountains, except on the border with Portugal, favors winds penetrating from the Atlantic Ocean and prevents or greatly reduces air circulation to and from the Bay of Biscay in the North of Spain and the southern plateau.

At present, Valladolid is one of the most polluted areas of the Castilla and León region. The thermal differences are notable even in the various streets related to parks, gardens, paved ground or not, height of buildings, industries, etc. [16]. The average temperature is about 8˚C at the end of winter and 13˚C in spring.

Five strategic points were considered: Poniente, Arco Ladrillo, La Rubia, Vega Sicilia and Valladolid Sur. A map of the Atmospheric Pollution Control Network of the Valladolid City Council (RCCAVA) [17] stations is shown in **Figure 1**.

Figure 2 shows the main characteristics of the five stations: Poniente station is in the downtown region in a parking area close to the Pisuerga river (average water flow 43 m^3/s at the study period). The Arco Ladrillo station is located next to the bus city general station and near the largest urban landmark. The La Rubia station is located within gardens, close to a central avenue with a large amount of road traffic. The Vega Sicilia station is in a residential area with several gardens. The Valladolid Sur station is located south of the city, next to an industrial zone near the VA-30 highway, with large, landscaped areas and undeveloped land. At the time of sampling, there was a civil construction, which could alter the movement and concentration of organic pollutants.

2.2. Bacterial Sample Collection and Analysis

Airborne bacterial spores were monitored from March to May 2019 in Valladolid, at 1.5 m height and at approximately 5 m from the measurement devices, to avoid the microorganism contamination from the soil degradation and to the closest source of environmental monitoring system from RCCAVA [18] [19].

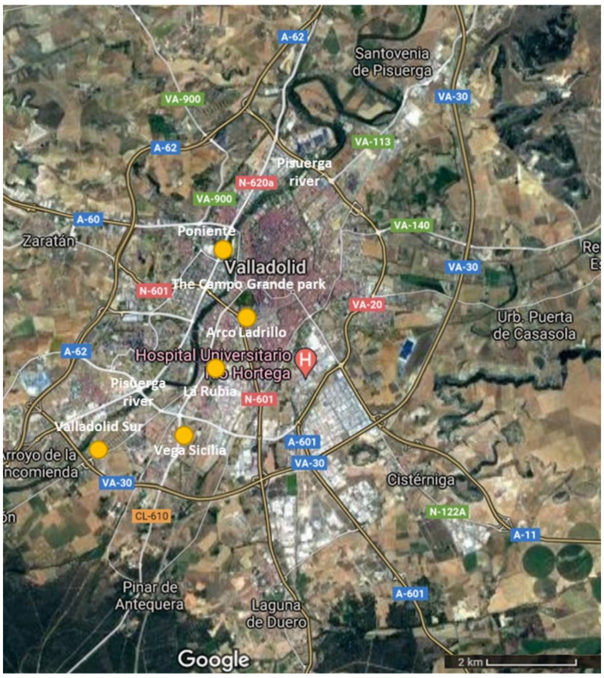

Figure 1. Aerial view of the city of Valladolid and distribution of the atmospheric pollution control network of the Valladolid City Council (RCCAVA) stations in Valladolid [17].

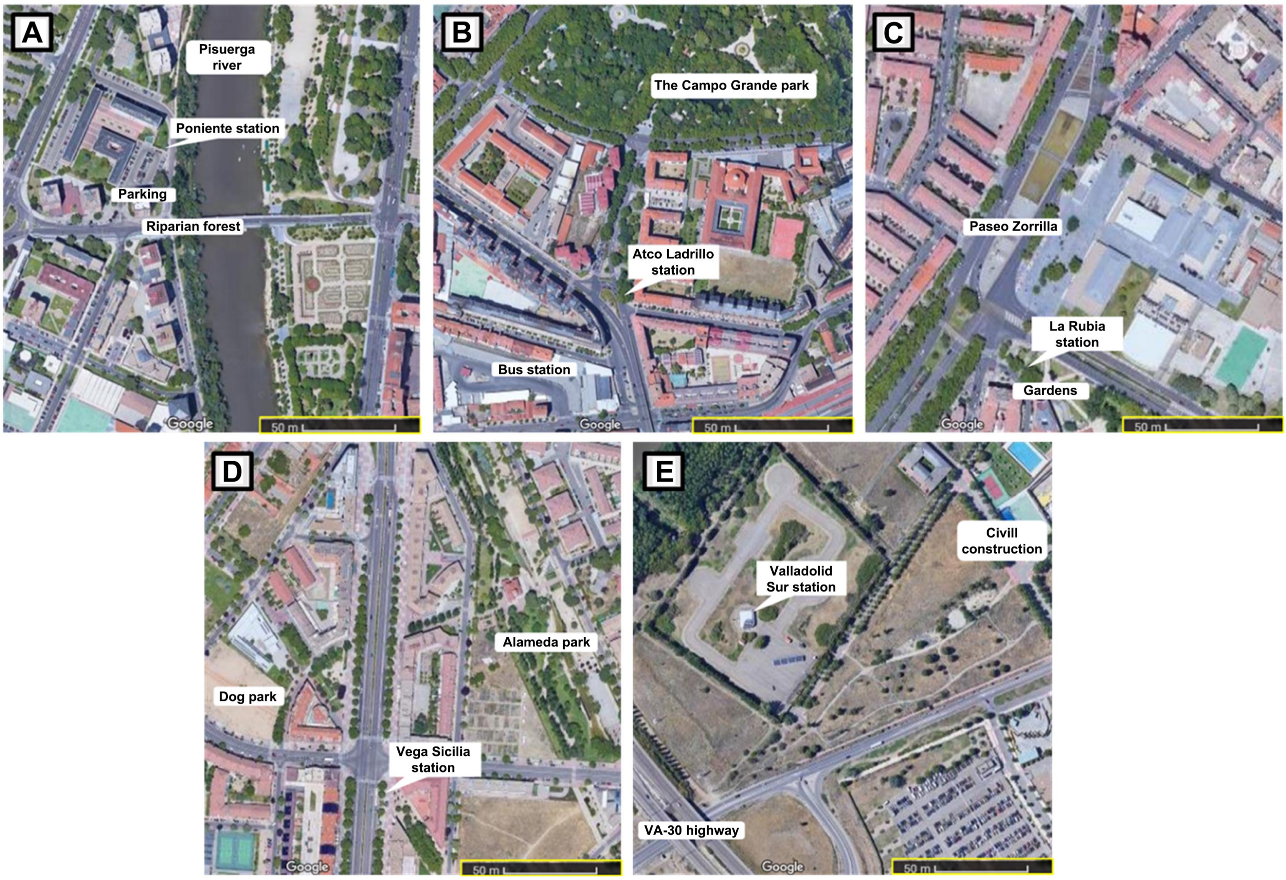

Figure 2. The RCCAVA stations in Valladolid [17]. (A) Poniente station, (B) Arco Ladrillo station, (C) La Rubia station, (D) Vega Sicilia station, (E) Valladolid-Sur station. Size of station surroundings: 50 m Google Maps scale.

Sample collection was carried out twice a month, at the same time in each geographical point between 8:00a.m. and 12:00p.m. The sampling interval was 8:00a.m.-9:00a.m. for Poniente station, from 9:00a.m.-10:00a.m. for Arco Ladrillo station, from 10:00a.m.-11:00a.m. for La Rubia station and from 11:00a.m.-12:00p.m. for Vega Sicilia and Valladolid Sur stations. Passive monitoring uses "settle plates", which are standard Petri dishes containing culture media, which are exposed to the air for 30 min to collect biological particles and are then incubated [20].

The culture media used to determine the presence of bacteria were selective culture of Trypticase Soy Broth (TSB), Mannitol Salt agar (Mannitol) and Standard Plate Count agar (PC) from Oxoid. The plates were quickly transferred to the laboratory and incubated in the dark, at 35˚C (TSB and Mannitol) or 25˚C (Potato Dextrose Agar and PC). Bacterial growth was carried out for 1 day and fungal growth for 4 days. For the characterization of the bacterial colonies, a gram stain was used [21] and visualized by optical microscope Olympus (model Cx41).

2.3. Data of Environmental Parameters

Atmosphere pollution and meteorological data were provided by the RCCAVA [17] and State Meteorological Agency (Aemet) [16]. The RCCAVA data collected correspond at the same temporal interval to when the sampling was carried out in each of the stations. The urban aerosol corresponds to a lightly industrialized city whose contamination mostly comes from road traffic and domestic heating. The economic activity is mainly relative to the automotive sector outside the city. Environmental parameters subjected to analysis included temperature, relative humidity, rainfall, and atmospheric pressure. Types of air pollution considered are sulfur dioxide (SO_2), carbon monoxide (CO), ozone (O_3), particulates with aerodynamic diameters of less than 10 mm ($PM10$) and 2.5 mm ($PM2.5$), nitrogen monoxide (NO) and nitrogen dioxide (NO_2).

3. Results

3.1. Characteristics of Ambient Bacterial Spores

Considering aerial microbial ecology and the difficulty to collect the same airborne microorganisms' spores at the different plates, we analyzed one plate in each interval of time for each station per geographical point. For the account of mesophilic bacteria, a colony forming unit (CFU) was used, equivalent to the number of bacteria observed on each agar plate. Sometimes many bacteria were observed that occupied a surface greater than a single colony, which were considered microbial sward. The confluence of several colonies hinders to determines an exact number of bacteria present. **Figures 3-5** show the cultures of ambient bacterial spores growing in the three media during the study period. A broad variety of bacteria and some fungi were observed. The geographic distribution of some microorganism's species depends on its environmental tolerance, dispersal

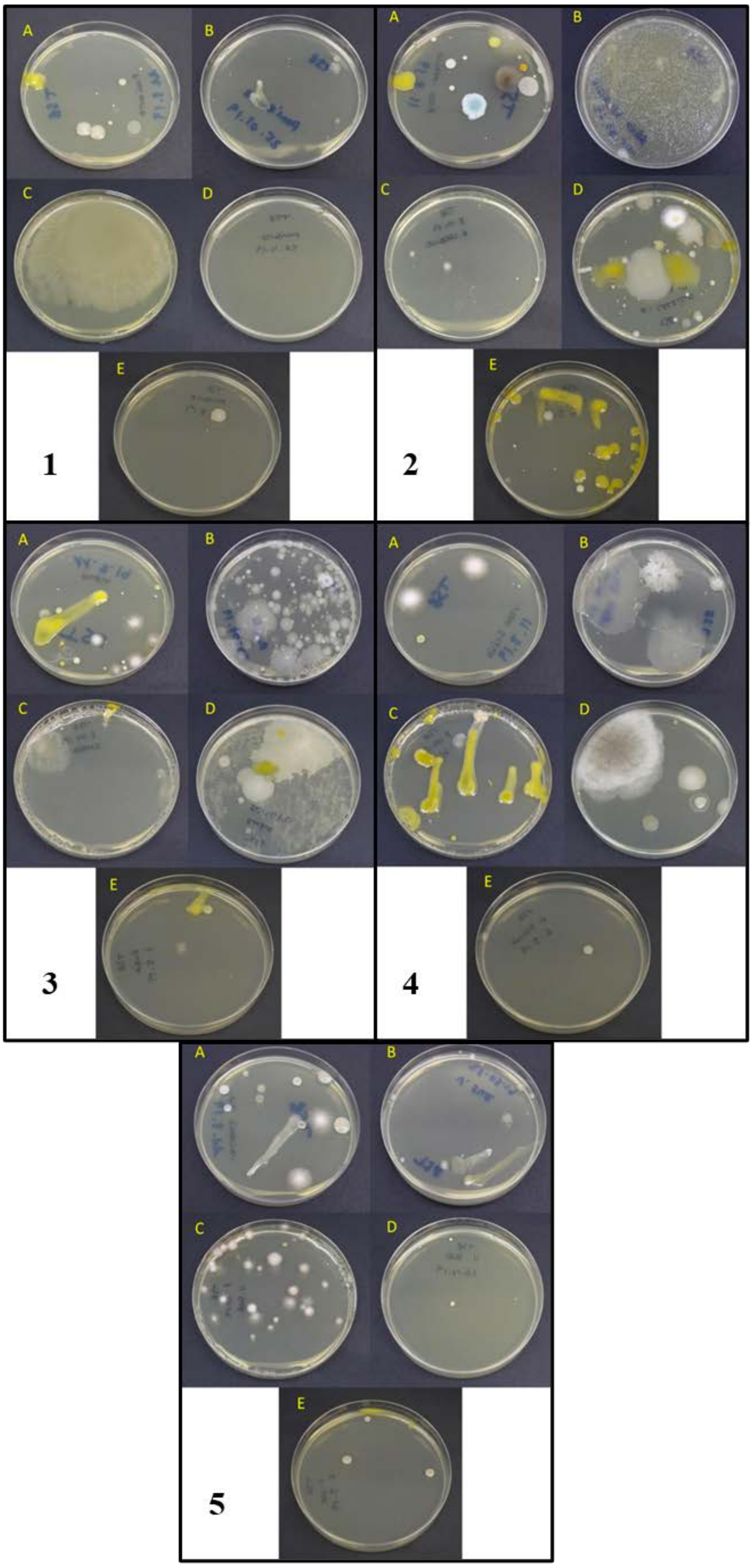

Figure 3. Bacterial growth at tryptic soy broth (TSB) medium on the sampling points of Poniente station (1), Arco Ladrillo station (2), La Rubia station (3), Vega Sicilia station (4) and Valladolid Sur station (5). (A) First sample; (B) second sample; (C) third sample; (D) fourth sample; (E) fifth sample.

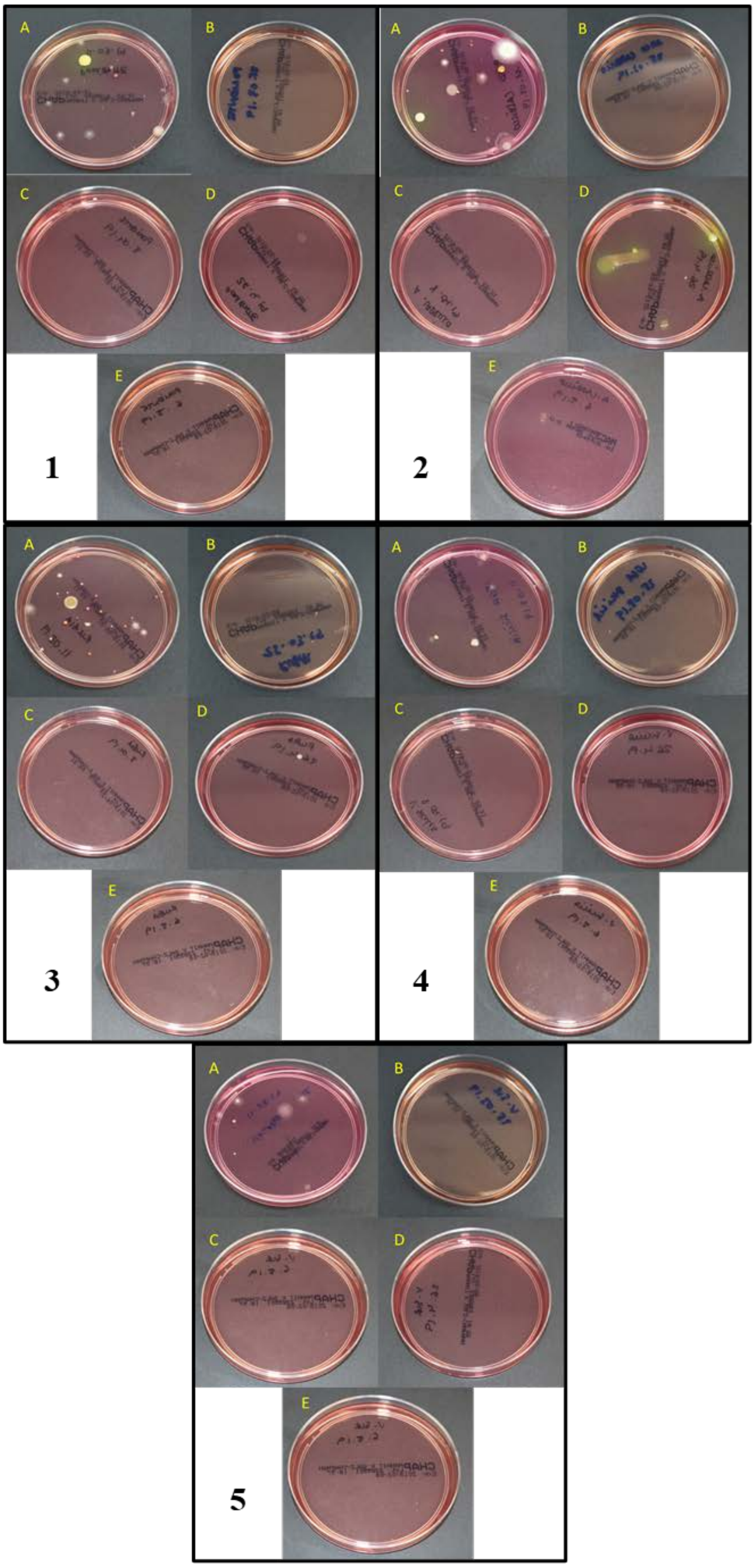

Figure 4. Bacterial growth at mannitol salt agar (Mannitol) medium on the sampling points of Poniente station (1), Arco Ladrillo station (2), La Rubia station (3), Vega Sicilia station (4) and Valladolid Sur station (5). (A) First sample; (B) second sample; (C) third sample; (D) fourth sample; (E) fifth sample.

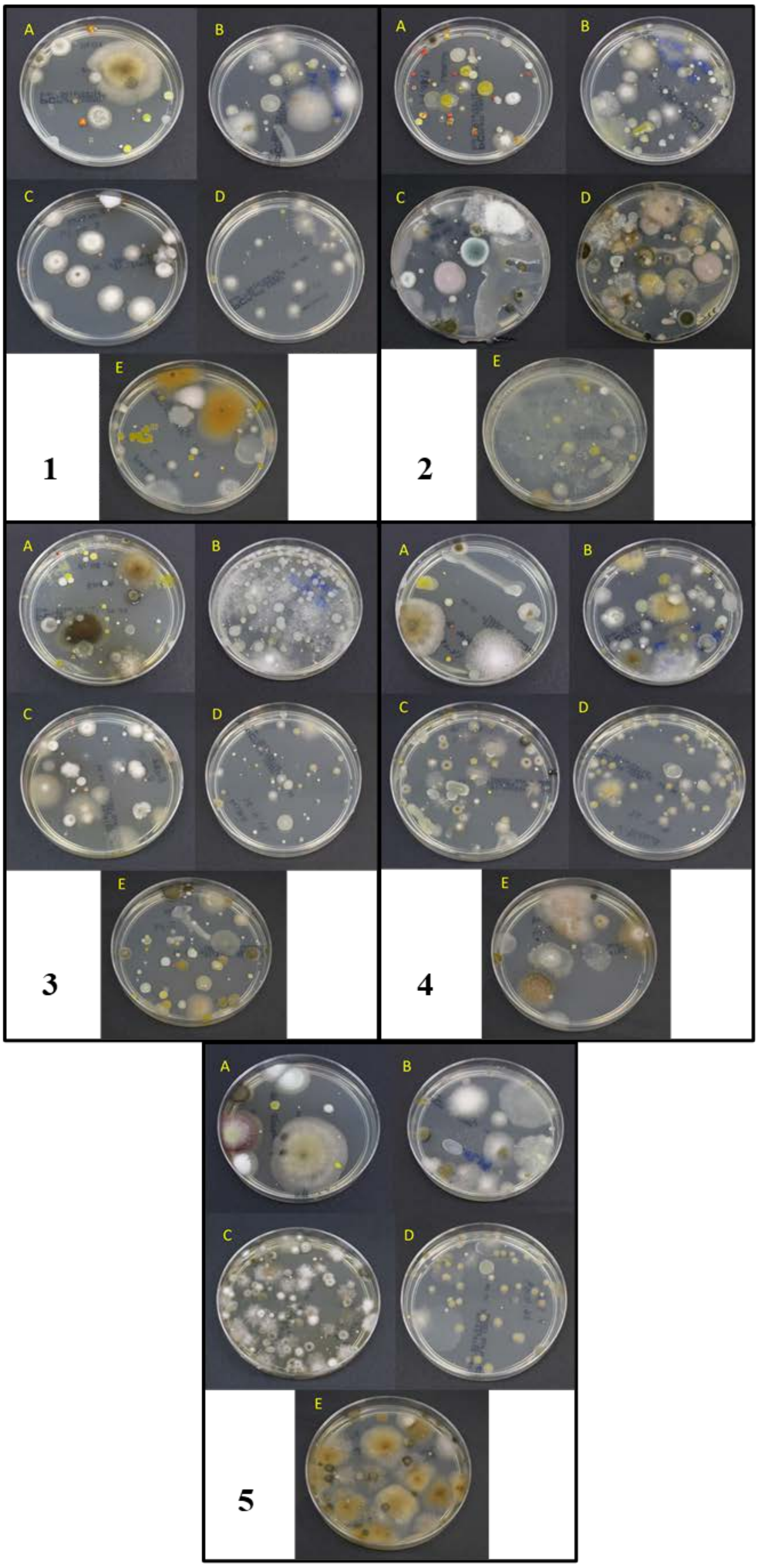

Figure 5. Bacterial growth at standard plate count agar (PC) medium on the sampling points of Poniente station (1), Arco Ladrillo station (2), La Rubia station (3), Vega Sicilia station (4) and Valladolid Sur station (5). (A) First sample; (B) second sample; (C) third sample; (D) fourth sample; (E) fifth sample.

constraints, and biological interactions with other species. The TSB medium has some selective agents for selective growth of *Staphylococcus aureus* and *Escherichia coli* (**Figure 3**). Mannitol, a selective medium for staphylococci and few halophilic species (**Figure 4**). In addition, fungal spores were detected in the three-culture media, but they were more abundant in the PC media (**Figure 5**).

Some bacterial colonies form flat, spreading colonies with a small twitching zone consisting of a very thin layer of cells which suggest cell motility. This occurs in a wide range of bacteria, depending on the presence of functional type IV fimbriae or pili [22].

The morphology observed under the microscope varies in selective media used (TSB, Mannitol and PC) between bacillary and coccoid clusters in the form of clusters or chains (**Tables S1-S3**). In general, the bacterial morphology that predominates in the atmosphere of Valladolid belongs to the cocci and bacilli, some of them belonging to pleomorphic and filamentous groups. Studies on the fungal spore presence in the air of Valladolid are currently being conducted.

Concerning the composition of cell wall, by gram staining, the predominant bacteria in the five sampling points of Valladolid are Gram-negative, but the proportion of Gram-positive and Gram-negative bacteria present in our samples varies depending on environmental conditions. Thus, for example, it was observed in TSB medium in the first, second and fourth samples collected in the station of Arco Ladrillo that the proportion of Gram-positive and Gram-negative bacteria are similar. However, in the second half of March and the first half of April at the Poniente station (2nd and 4th sampling), the proportion of Gram-positive bacteria also increased.

In Mannitol medium, considered as a specific medium for *Staphylococcus* genus, the presence of Gram-positive bacteria with a coccoid morphology was observed in the fourth sampling at La Rubia and Arco Ladrillo station. Considering the bacteria grown in the PC medium, we can conclude that the majority were Gram-negative genera, among which is the *Escherichia* genus. Some of them synthetize virulence factors that affect a wide range of cellular processes.

3.2. Environmental Parameters and Microorganisms

The temperature interval during the study period was between a minimum value of 8˚C and a maximum value of 18˚C in May. Wind speed was higher in the second week of April, reaching 4.7 m/s. No precipitation was observed during the samplings, but it was observed during the days prior to the second week of April.

The ozone measurement was collected in the Poniente, Vega Sicilia and Valladolid Sur stations. **Table 1** shows that in the second sampling in the Valladolid Sur station its concentration was higher (100 $\mu g/m^3$), followed by Poniente. In the third sample, its concentration was higher in this season, and in the fourth sample it was lower, being the lowest value in Vega Sicilia (40 $\mu g/m^3$). Vega Sicilia shows its highest concentration in the third sample.

Table 1. Air pollutant concentrations and weather conditions that existed at the time of sampling. Values are taken from RCCAVA average concentrations to the temporal interval corresponding to when the sampling was carried out in each of the stations [17].

Station	Sample	Temp. (˚C)	Relative Humidity (%)	Wind (m/s)	CO (mg/m³)	NO (µg/m³)	NO_2 (µg/m³)	0_3 (µg/m³)	*PM*10 (µg/m³)	*PM*2.5 (µg/m³)	SO_2 (µg/m³)
Poniente	1	8.9	60	1.7	N/A	1.5	7.5	72	12	9	N/A
	2	9	53	2.1	N/A	1.5	9	91	29	20	N/A
	3	9.7	75	3.8	N/A	6	10	87.5	N/A	N/A	N/A
	4	10	65	4.3	N/A	7.5	12	71.5	9,9	N/A	N/A
	5	11.4	70	2.3	N/A	9	20.5	69.5	13	4.5	N/A
Arco Ladrillo	1	10.3	60	1.8	0.6	10.5	23.5	N/A	17	4	N/A
	2	10.9	53	2.4	0.3	10	17.5	N/A	19,5	N/A	N/A
	3	10.8	75	3.9	0.4	3.5	10.5	N/A	9,5	2.5	N/A
	4	11.2	65	3.7	0.4	5	15	N/A	13,5	5	N/A
	5	12.6	70	2.5	0.55	N/A	N/A	N/A	18,5	7	N/A
La Rubia	1	11.3	60	1.8	N/A	13	19.5	N/A	8.5	8	1
	2	12.4	53	2.4	N/A	10	16	N/A	22.5	N/A	3
	3	11.5	75	3.7	N/A	7.5	15	N/A	14.5	6.5	4.5
	4	12.1	65	3.4	N/A	12,5	14.5	N/A	12.5	6	3
	5	14.1	70	2.4	N/A	13	23.5	N/A	21	11.5	5
Vega Sicilia	1	12.3	60	1.9	N/A	9	15	74	13	7.5	N/A
	2	13.5	53	2.5	N/A	N/A	N/A	93.5	26	16	N/A
	3	12.4	75	3.8	N/A	N/A	N/A	98.5	7	N/A	N/A
	4	13.3	65	3.5	N/A	3.5	5	40	4	2.5	N/A
	5	16.6	70	2.6	N/A	1.5	3	90	8	8	N/A
Valladolid Sur	1	12.3	60	1.9	N/A	1	2.5	19.5	N/A	N/A	N/A
	2	13.5	53	2.5	N/A	2	1.5	100	N/A	N/A	N/A
	3	12.4	75	3.8	N/A	3	4.5	88.5	N/A	N/A	N/A
	4	13.3	65	3.5	N/A	1	4.5	75.5	N/A	N/A	N/A
	5	16.6	70	2.6	N/A	1	8	89.5	N/A	N/A	N/A

N/A, Not available.

Carbon monoxide (CO) was only collected in the Arco Ladrillo station (see Table 1). Possibly the city hall authorities considered taking these values into account in the area where road traffic was greater in the city.

As pollutants, nitrogen oxides (NOx), mainly anthropic in origin, are gases that are emitted in combustion processes depending on car traffic. The highest levels of NOx are reached in large urban agglomerations, such as the La Rubia and Arco Ladrillo stations, which are metropolitan areas with denser traffic. Such is the case of the La Rubia and Arco Ladrillo stations. In Table 1, we compare temperature and NOx, and find an increase in its value with temperature and a decrease in wind values.

Regarding particulate material, it should be noted that it has been measured in the urban nucleus of the city of Valladolid, and not in residential areas. A direct relationship with the NOx concentration cannot be observed. A more constant measurement by government authorities would be necessary.

High concentrations of SO_2 in the air generally also lead to the formation of other sulfur oxides (SOx). SOx can react with other compounds in the atmosphere to form small particles. These particles contribute to particulate matter (*PM*) pollution. Small particles may penetrate deeply into the lungs and in sufficient quantity can contribute to health problems [23]. It would be of great interest in terms of human health to know this value for all the RCCAVA stations of the city [17].

4. Discussion

In this study, the presence of airborne microorganisms in the atmosphere of the city of Valladolid was analyzed. The prevalence of allergic sensitization at Valladolid corresponds to the period from March to May, previously this period was also considered by Lâm *et al.* (2014) [24]. The study examines the impacts of climate-driven microorganisms species redistribution on ecosystem health, and human well-being. The climate of Valladolid is determined by the location of the city in the center of the sedimentary plateau of the Duero, almost surrounded by mountains that isolate it from the sea. The city has an extreme and dry climate. Rainfall is distributed irregularly throughout the year, although there is a minimum in the summer and a maximum in autumn and spring. The pollution levels are strongly dependent on the atmospheric synoptic conditions, in this case a long and cold winter, with frequent fog events. The proportion, morphology and type of bacterial genus that grew in four general and specific culture media. In the sampling, the abundance and diversity of microorganisms at the air of the city have been different; it was observed that the number of bacteria that grew was greater in the La Rubia station. At the Poniente station, a lower proportion of bacteria was found in the sampling.

In general, sample collection of the bacteria showed some tendency toward the loss of urban airborne biodiversity, consequence of the stressor's factors such as temperature, dryness and fragmented habitat present at the atmosphere city.

When the ozone concentration increased, in the second and third samples, there was no bacterial growth for Mannitol salt agar. Ozone inhibited the growth of bacterial strains, as previously described by Fontes *et al.* [25]. Likewise, when the concentrations of NOx increased, the presence of bacteria of the *Staphylococcus* genus in the Mannitol medium was observed in Arco Ladrillo, Vega Sicilia and Valladolid-Sur stations. In the PC medium, the highest number of bacteria was observed in the second sample and the lowest number in the third sample. This fact was accompanied by an increase in the concentration of ozone and lower levels of UV light.

The photoautotrophic microorganisms are present in the lower troposphere of the atmosphere. In industrial areas there may even be a sufficient concentration of organic substances in the atmosphere to support the growth of some heterotrophic microorganisms [4]. It is accepted that the concentration of microorganisms in air can vary depending on the meteorological seasons and geographic locations. In addition, in industrial areas, or by road traffic, there may be an adequate concentration of organic substances in the atmosphere that allow the growth of some heterotrophic microorganisms. Some of these microorganisms are vegetative cells, allowing them to survive the atmosphere restrictive conditions such as high levels of UV radiation, desiccation (wind drying) and temperature and atmospheric chemistry. The great adaptability of aerial microorganisms makes them capable of proliferating in any type of environment, whether it is near an industrial center, in the city center or in a green area on the outskirts of the urban nucleus [26] [27]. Also, the results shown a positive correlation between temperature and aerobic mesophilic bacteria concentration in the urban atmosphere, previously observed by Monteiro *et al.* (2021) [28]. Most bacteria in our samples were Gram-negative, which have lower resistance to physical destruction or desiccation, compared with Gram-positive bacteria [29].

Previous studies on air quality carried out in the city of Valladolid show that the particulate matter found in the atmosphere is related to cardiovascular diseases [30], which confirms previous works [31]. We can find microorganisms in aerosol particles, and they can develop because of the water present in the clouds, in addition to the concentrations of carbon dioxide linked to the light intensity reflected from the soil, water and living beings, like previous studies [32]. When we compared the collected data by RCCAVA [17] with the number of bacteria grown in the PC medium, the highest number was observed in the second sampling when the $PM10$ values were higher. Of all the stations where samples were collected, La Rubia had the greatest amount of bacteria growth, a fact supported by studies in which it is considered that the presence of high buildings allows them to proliferate.

5. Conclusions

The types of bacteria present in the atmosphere of Valladolid can be driven by differences in bacterial sources of the atmosphere and a wide range of environmental factors, including UV intensity, precipitation events and humidity. Our results indicate that, generally, Gram-negative bacteria predominate over Gram-positive bacteria; and significant decrease in Gram-positive bacteria is observed between the months of March and May.

There is not a clear direct relationship between the concentrations of atmospheric pollutants and the presence of airborne microorganisms in the areas monitored by a network of stations (RCCAVA) [17]. Similarly, and as expected, the concentration of pollutants is directly related to meteorological conditions like wind, temperature, or rain. Bacteria increase with the wind. Humidity scavenges particles and particle-associated bacteria from the air.

The presence of certain bacterial types is of interest, not only for their ability to mediate a range of chemical and physical processes in the atmosphere, such as cloud formation and ice nucleation, but also for their beneficial or detrimental implications for human health. Knowledge of the transportation of bacteria by air currents can aid in the prevention of the airborne spread of pathogens or source identification.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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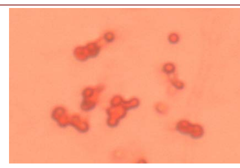
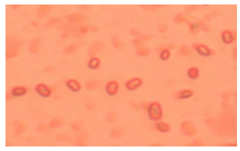
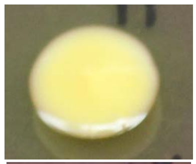
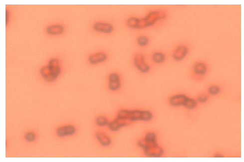
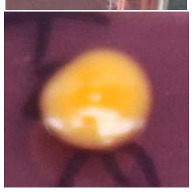
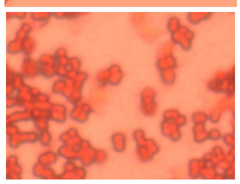
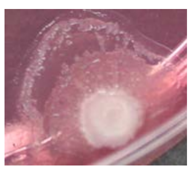
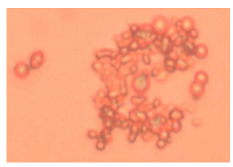
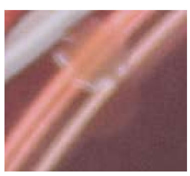
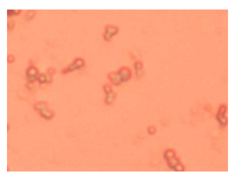
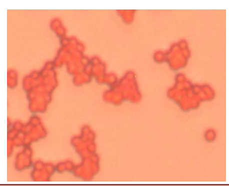
Supplementary

Table S1. Macroscopic view of colony grow up at Mannitol Salt Agar (Mannitol) medium on the five sampling points with their morphology. The bacteria morphology images were made with a magnification of ×60.

Macroscopic view of colony	Bacteria morphology	Sampling points collected
		Poniente (1st) Arco Ladrillo (1st) La Rubia(1st) Vega Sicilia (1st)
		Poniente (1st) Arco Ladrillo (1st) La Rubia (2nd) Vega Sicilia (2nd)
		Poniente (1st) Arco Ladrillo (1st) Arco Ladrillo (4th)
		Poniente (1st) Vega Sicilia (1st)
		Poniente (1st)
		Arco Ladrillo (1st)
		Arco Ladrillo (1st) Vega Sicilia (1st)
		Arco Ladrillo (1st) La Rubia (2nd)
		Arco Ladrillo (1st) Vega Sicilia (1st)

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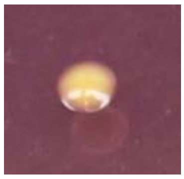
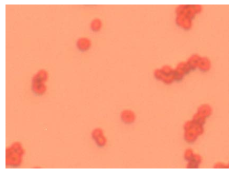
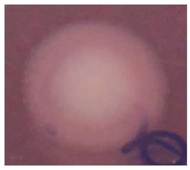
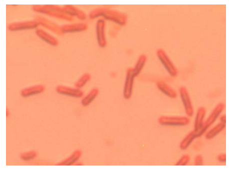
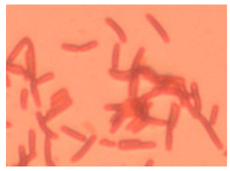
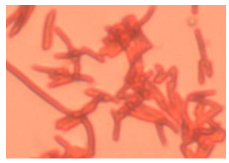
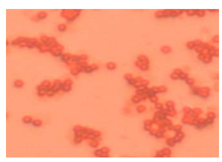
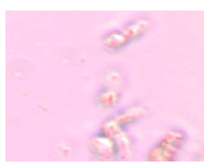
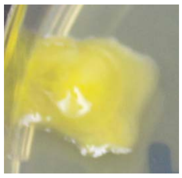
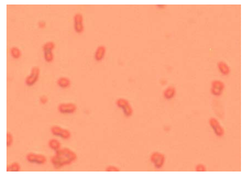
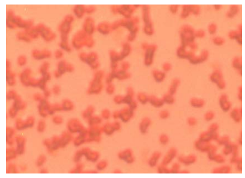
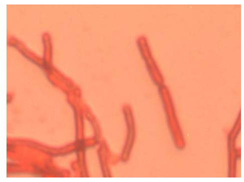
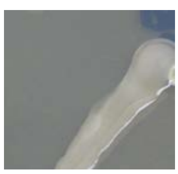
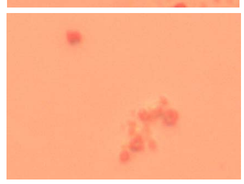
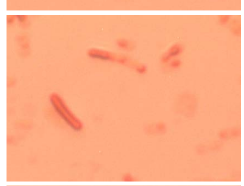
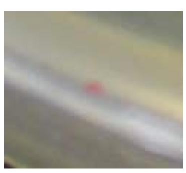
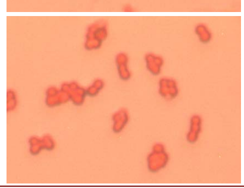
		Valladolid Sur (1st) Vega Sicilia (1st)
		Valladolid Sur (1st)
		La Rubia (1st)
		La Rubia (1st)
		La Rubia (2nd)
		Valladolid Sur (2nd) Arco Ladrillo (1st) La Rubia (2nd)
		Poniente (4th)
		La Rubia (4th) Arco Ladrillo (4th)
		Poniente (1st, 5th) Arco Ladrillo (2nd) La Rubia (1st)

Table S2. Macroscopic view of colony grow up at trypticaseinsoy broth (TSB) medium on the five sampling points with their morphology. The bacteria morphology images were made with a magnification of ×60.

Macroscopic view of colony	Bacteria morphology	Sampling points collected
		Poniente (1st) Arco Ladrillo (1st, 4th, 5th) La Rubia (1st, 3rd, 4th, 5th) Vega Sicilia (3rd) Valladolid-Sur (5th)
		Poniente (1st) Arco Ladrillo (1st)
		Poniente (1st) Arco Ladrillo (1st) Valladolid-Sur (1st)
		Poniente (1st) La Rubia (2nd) Arco Ladrillo (3rd) Vega Sicilia (4th)
		Poniente (1st, 2nd) La Rubia (2nd, 4th) Arco Ladrillo (4th) Vega Sicilia (4th) Valladolid-Sur (1st, 2nd)
		Arco Ladrillo (2nd) La Rubia (1st) Vega Sicilia (1st) Valladolid-Sur (1st, 3rd)
		Arco Ladrillo (4th) La Rubia (3rd) Vega Sicilia (2nd, 3rd) Valladolid-Sur (1st, 2nd)
		Valladolid-Sur (1st)
		Arco Ladrillo (1st) La Rubia (1st) Vega Sicilia (1st)

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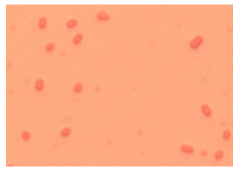
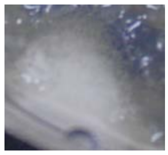
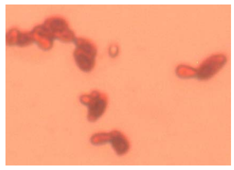
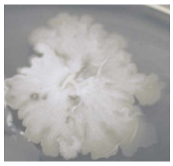
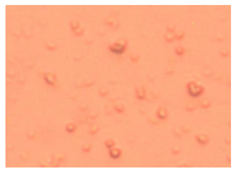
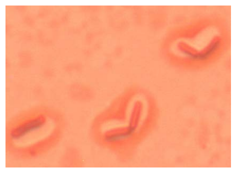
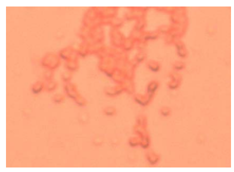
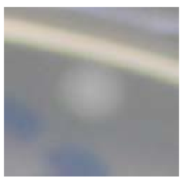
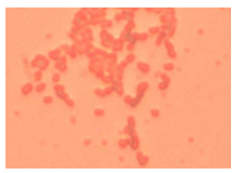
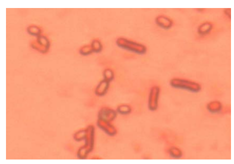
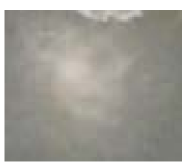
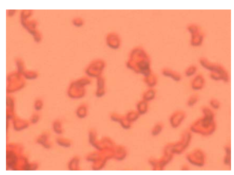
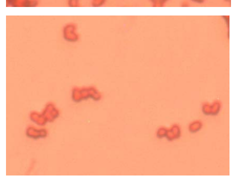
		Arco Ladrillo (1st) Poniente (5th)
		Poniente (2nd)
		Arco ladrillo (2nd)
		Poniente (2nd) Vega Sicilia (2nd)
		La Rubia (2nd) Vega Sicilia (2nd)
		Arco Ladrillo (2nd) Valladolid-Sur (2nd)
		Valladolid Sur (2nd)
		Valladolid Sur (2nd) Arco Ladrillo (1st)
		La Rubia (3rd) Arco Ladrillo (1st, 3rd) Valladolid-Sur (3rd)
		Arco Ladrillo (1st, 3rd, 5th) Poniente (1st)

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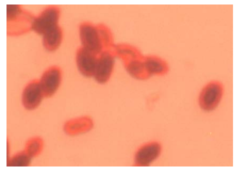
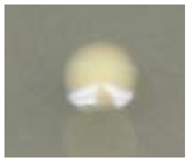
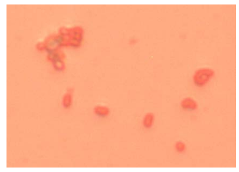
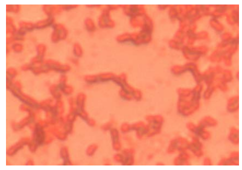
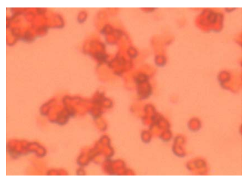
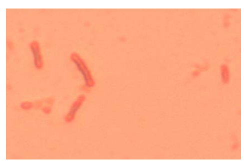
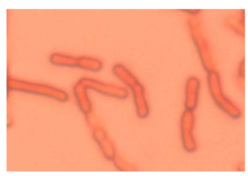
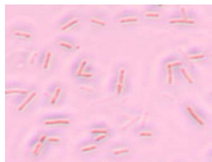
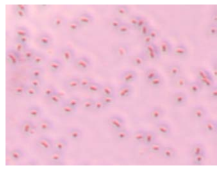
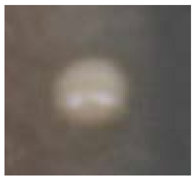
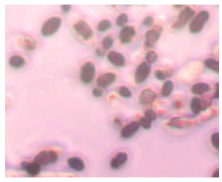
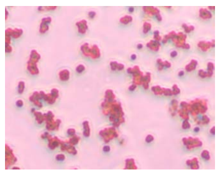
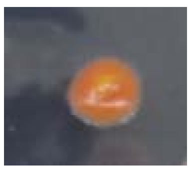
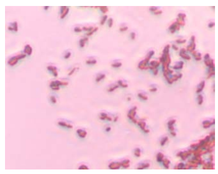
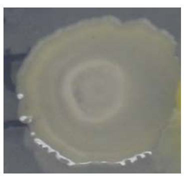
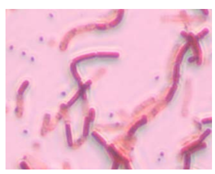
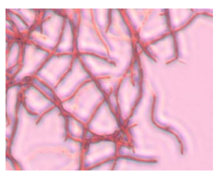
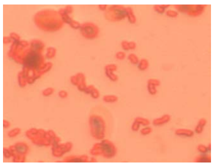
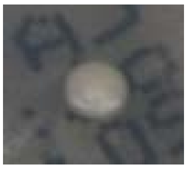
		Poniente (2nd, 5th) Arco Ladrillo (3rd, 4th) La Rubia (1st, 3rd) Vega Sicilia (1st) Valladolid-Sur (2nd)
		Arco Ladrillo (4th, 5th) Poniente (1st) La Rubia (1st, 2nd) Vega Sicilia (1st, 4th) Valladolid-Sur (4th)
		Poniente (4th) La Rubia (4th, 5th) Valladolid-Sur (1st)
		Poniente (1st) Valladolid-Sur (3rd, 5th)
		Valladolid-Sur (5th)
		Arco Ladrillo (5th)
		La Rubia (5th)
		Arco Ladrillo (4th, 5th) La Rubia (2nd, 4th) Vega Sicilia (3rd, 4th, 5th) Valladolid-Sur (2nd, 5th)

Table S3. Macroscopic view of colony grow up at standard plate count agar (PC) medium on the five sampling points with their morphology. The bacteria morphology images were made with a magnification of ×60.

Macroscopic view of colony	Bacteria morphology	Sampling points collected
		Poniente (3rd) La Rubia (3rd) Valladolid-Sur (1st) Vega Sicilia (2nd)
		Arco Ladrillo (1st, 2nd, 3rd, 4th, 5th) Poniente (1st, 4th, 5th) La Rubia (1st, 2nd, 3rd, 4th, 5th) Vega Sicilia (1st, 2nd, 3rd, 4th, 5th) Valladolid-Sur (1st, 2nd, 3rd, 4th, 5th)
		Poniente (1st, 2nd, 3rd, 4th) Arco Ladrillo (1st, 2nd, 3rd, 4th) La Rubia (1st, 2nd, 3rd, 4th, 5th) Vega Sicilia (1st, 2nd, 3rd, 4th, 5th) Valladolid-Sur (1st, 2nd, 3rd, 4th)
		Arco Ladrillo (5th) Valladolid-sur (1st, 5th)
		Poniente (5th) Arco Ladrillo (1st, 5th) La Rubia (1st, 5th) Vega Sicilia (1st)
		Poniente (1st, 2nd, 3rd) Arco Ladrillo (1st, 3rd) La Rubia (3th, 4th) Vega Sicilia (5th)
		Poniente (2nd) Arco Ladrillo (1st) La Rubia (1st, 2nd) Vega Sicilia (1st, 2nd) Valladolid Sur (2nd)
		Vega Sicilia (1st)
		Poniente (2nd, 5th) La Rubia (5th)

Continued

		Poniente (5th) Arco Ladrillo (2nd, 4th) La Rubia (5th)
		Arco Ladrillo (2nd) La Rubia (2nd, 5th) Vega Sicilia (1st, 2nd) Valladolid-Sur (5th)