Direct Colony Thin-Layer Chromatography and Rapid Characterization of *Serratia marcescens* Mutants Defective in Production of Wetting Agents

TOHEY MATSUYAMA,* MASAKAZU SOGAWA, AND IKUYA YANO†

Department of Bacteriology, Niigata University School of Medicine, Niigata 951, Japan

Received 8 August 1986/Accepted 10 February 1987

A bacterial mass (ca. 1 mg) was placed directly on a thin-layer chromatography plate and developed shortly in chloroform-methanol (2:1, vol/vol). After being dried, the bacterial mass was developed in chloroform-methanol-5 M ammonia (80:25:4, vol/vol). The obtained chromatogram indicated the characteristic lipid compositions of the bacteria. So, it became possible to examine bacterial colonies at once for the identification of mutants defective in the production of specific lipids.

Thin-layer chromatography (TLC) is a useful method for the analysis of bacterial lipids. Although TLC itself is fast and requires no special equipment, it is cumbersome, and it takes several days to prepare lipid samples for TLC. During studies of *Serratia marcescens*, which was shown to have wetting activity via the production of specific aminolipids (2, 3), we invented a simple method for the rapid examination of such bacterial lipids. By applying a bacterial colony mass directly on a TLC plate, we found it possible to characterize the main lipid composition of the bacteria.

S. marcescens strains were described previously (3). Mutant strains NS25-03, NS38-09, and NS38-12 were obtained by UV irradiation. *Serratia liquefaciens* ATCC 27592, NCTC 10422, and CDC 1284-57 and *Serratia rubidaea* ATCC 27593 and CDC 299-72 were supplied by E. Yabuuchi, Gifu University School of Medicine. *S. rubidaea* WH-05 was a spontaneous mutant of *S. rubidaea* ATCC 27593. *Escherichia coli* ATCC 25922 and W3350/1 were laboratory stocks. These strains were grown at 30°C, with the exception of *S. rubidaea* CDC 299-72, which was grown at 27°C, for 3 days on peptone-glycerol agar (4).

As described previously (2), wetting activity was examined by mixing a small amount of bacterial mass with a drop of water on a clean glass slide. If the bacteria had wetting activity, rapid and spontaneous spreading of the bacterial suspension was observed on the glass surface. Wetting activity was also recognized by placing 10 µl of a bacterial suspension on a water-repelling material (cotton). A suspension of *S. marcescens* NS38 (wild type) wetted the material with ease, whereas that of mutant NS38-09 remained as a drop and did not sink into the material (Fig. 1). Thus, wetting activity seemed to be important in bacterial translocation behavior on various surfaces.

In our new TLC method, a bacterial mass (ca. 1 mg) as large as one colony was placed on a Silica Gel G TLC plate (Analtech, Newark, Del.) and predeveloped in chloroform-methanol (2:1, vol/vol) for 10 min. After 15 min of drying, the shrunken bacterial mass adhering to the plate was removed. Bacterial extracts remaining on the plate were developed in chloroform-methanol-5 M ammonia (80:25:4, vol/vol). Se-

quential examination with 0.2% (wt/vol) ninhydrin-ethanol solution, Dittmer reagent (1), and 50% H₂SO₄ was carried out to characterize developed spots.

Chromatograms obtained by the direct colony TLC and the ordinary TLC are compared in Fig. 2. The new method revealed the same characteristic lipid composition of *S. marcescens* NS25 (lane 2) as did the ordinary method (lane 3). So, without ordinary procedures of lipid extraction from each bacterial culture (3), it was possible to examine the lipid compositions of many bacterial strains at once.

In previous studies (2, 3), we found specific aminolipids (W1, W2, and W3) in *S. marcescens* cultures demonstrating wetting activities. Dispersions of these isolated aminolipids also demonstrated strong wetting activities on a glass surface and made small contact angles on a polystyrene surface. Thus, these aminolipids were considered to be wetting agents produced by *S. marcescens* strains. W1 was the wetting agent produced by NS38 and ATCC 13880 strains and was identified as a cyclodepsipeptide identical to seratamolide (2). Screening of *S. marcescens* NS38 mutants for defectiveness in the production of this wetting agent was carried out by our direct colony TLC (Fig. 3). It is evident that the bacterial colony in lane 9 of the chromatogram is the mutant lacking the wetting agent. The mutant thus obtained, NS38-09, demonstrated no wetting activity on a glass slide or on cotton (Fig. 1). In the same way, it was easy to identify

FIG. 1. Wetting activity of *S. marcescens*. Bacterial suspensions (10 µl) of wild-type NS38 (1) and mutant NS38-09 (2) were placed on cotton.

* Corresponding author.

† Present address: Department of Bacteriology, Osaka City University School of Medicine, Osaka 545, Japan.

FIG. 2. Thin-layer chromatograms obtained by three different ways in chloroform-methanol-5 M ammonia. Lanes: 1, bacterial mass of *S. marcescens* NS25; 2, the same bacterial mass developed beforehand in chloroform-methanol; 3, 50 µg of lipids extracted from the same bacterial strain by the ordinary extraction method. The plate was sprayed with 50% H₂SO₄ and heated at 200°C for 20 min. W2, Wetting agent of this strain; UG, unidentified glycolipids; PL, phospholipids; NP, ninhydrin-positive unidentified lipids.

mutants of *S. marcescens* NS25 and *S. rubidaea* ATCC 27593. Specific spots corresponding to wetting agents (W2 and RG1) were absent in these mutants (Fig. 4, lanes 2 and 11). RG1 was a glycolipid produced by *S. rubidaea* strains and had wetting activity by itself (T. Matsuyama et al.,

FIG. 3. Mutant-screening chromatograms obtained by the direct colony TLC. A bacterial mass from each colony of UV-treated *S. marcescens* NS38 was examined (lanes 1 to 11). P, Prodigiosin; W1, wetting agent (serratamolide); PL, phospholipids; OL, ornithine lipids.

FIG. 4. Direct colony thin-layer chromatograms of *S. marcescens* NS25 (lane 1), NS25-03 (lane 2), NS38 (lane 3), NS38-09 (lane 4), NS38-12 (lane 5), and ATCC 13880 (lane 6); *S. liquefaciens* ATCC 27592 (lane 7), NCTC 10442 (lane 8), and CDC 1284-57 (lane 9); *S. rubidaea* ATCC 27593 (lane 10), WH-05 (lane 11), and CDC 299-72 (lane 12); and *E. coli* ATCC 25922 (lane 13) and W3350/1 (lane 14). P, Prodigiosin; W1 and W2, wetting agents of *S. marcescens*; UL, unidentified lipids of *S. rubidaea*; PL, phospholipids; NP, ninhydrin-positive unidentified lipids; RG, wetting agent (glycolipid) of *S. rubidaea*.

manuscript in preparation). In addition to differences between a wild type and a mutant, characteristic lipid compositions of bacterial species were demonstrated (Fig. 4) in a short time (2 h).

FIG. 5. Two-dimensional chromatogram of lipids of *S. marcescens* NS38 cultivated at 37°C. The first development was carried out by the direct colony method. O, Origin; 1, phosphatidylglycerol; 2, ornithine lipid; 3, phosphatidylethanolamine; 4, unidentified lipid; 5, cardiolipin; 6, W1 wetting agent.

For further separation and characterization of bacterial lipids, the direct colony TLC followed by two-dimensional TLC in chloroform-methanol-water (65:25:4, vol/vol) was performed. Separated spots of phosphatidylethanolamine, phosphatidylglycerol, cardiolipin, and ornithine lipid of *S. marcescens* can be seen in Fig. 5.

In this direct colony TLC, lipids in a bacterial mass placed on a TLC plate were extracted in an ascending flow of chloroform-methanol. Evaporation of the extraction solvent and exclusion of insoluble materials were simply done by drying the plate and removing the shrunken bacterial mass. Since extracted lipids were developed in situ in subsequent solvent systems, decomposition or loss of the extracts seemed negligible. Thus, with a small amount of culture,

examination of microbial lipids might be possible in a short time.

LITERATURE CITED

1. Dittmer, J. C., and R. L. Lester. 1964. A simple specific spray for detection of phospholipids on thin layer chromatograms. *J. Lipid Res.* **5**:126-127.
2. Matsuyama, T., M. Fujita, and I. Yano. 1985. Wetting agent produced by *Serratia marcescens*. *FEMS Microbiol. Lett.* **28**:125-129.
3. Matsuyama, T., T. Murakami, M. Fujita, S. Fujita, and I. Yano. 1986. Extracellular vesicle formation and biosurfactant production by *Serratia marcescens*. *J. Gen. Microbiol.* **132**:865-875.
4. Morrison, D. A. 1966. Prodigiosin synthesis in mutants of *Serratia marcescens*. *J. Bacteriol.* **91**:1599-1604.