Effects of Growth Media Composition on Colonial Morphology and Sliding Motility in the Soil Bacterium, *Bacillus cereus*

Karlie C. Brown and Lawrence R. Aaronson
Biology Department, Utica College, Utica, NY

ABSTRACT

Bacillus cereus is a bacterium found in soil and as a contaminant of unpasteurized milk and dairy products, causing gastrointestinal illness if ingested. Under standard laboratory conditions on a variety of agar culture media, *B. cereus* grows as spreading colonies with smooth scalloped edges. Several years ago, we observed that when grown on milk agar, *B. cereus* forms colonies with unusual ragged, dendritic margins. Microscopic analysis of colonies revealed stringy and swirling outgrowths of bacteria from the main colony mass. This pattern is indicative of a distinct means of bacterial motility known as sliding. We hypothesized that the only reason sliding motility is observed on milk agar is due to the hydrophobic nature of the milk protein, casein, which changes the surface tension of the agar. We tested this hypothesis by growing *B. cereus* on agar containing tryptone (partially digested casein) and on casamino acids (CAA, acid hydrolyzed casein) supplemented with the nonionic detergent Tergitol NP40. We observed sliding motility on tryptone + NP40, though far less sliding on CAA + NP40, supporting the hypothesis that *B. cereus* senses changes in surface tension on agar medium and alters its mode of motility. Addition of glucose to media with tryptone + NP40 does not eliminate sliding morphology, suggesting that induction of sliding motility is not a response to nutrient starvation. The addition of glucose to CAA media induces a change in colonial morphology when compared to tryptone media. The pH of growth media has a profound effect on sliding motility: under acidic conditions (pH 6.0), long, stringy sliding structures at colonial margins are abundant. As pH increases into the neutral range, the stringy structures shorten and become swirling "van Gogh structures", and sliding is absent at pH 8.0. In order to examine the role of bacterial motility on colony formation, we used time-lapse photography to observe early colony development on tryptone agar with and without NP40. As expected, NP40 promotes sliding motility and colony spreading from the outset. On tryptone agar alone, however, stringy sliding structures are evident early in colony growth, but develop into van Gogh structures and build colonies vertically through swarming as opposed to laterally by sliding. Results indicate that several environmental conditions, including hydrophobicity/surface tension and pH, affect the induction of sliding motility and alteration of colonial morphology in *Bacillus cereus*.

BACKGROUND

Under standard laboratory conditions and on a variety of plate media, *Bacillus cereus* exhibits swarming motility. Swarming *B. cereus* is characterized by colonies that have a crenulated surface with smoothly scalloped colony margins. Several years ago, we observed a characteristically different type of colonial morphology when *B. cereus* was grown on litmus milk agar plates. On milk agar, *B. cereus* colonies produce stringy outgrowths and swirling columns at their margins. This colonial morphology is consistent with a type of bacterial motility known as sliding. Sliding bacterial cells lose their flagella and push themselves away from the main colony mass via reproduction, sliding on a layer of surfactant (1, 2). Sliding motility is well studied in other *Bacillus* species, such as *Bacillus subtilis* (3) but little is known about the mechanism that causes the switch from swarming to sliding motility in *B. cereus*.

We hypothesized that sliding motility on milk agar was due to the presence of the hydrophobic milk protein, casein. To test this, we grew *B. cereus* on tryptone (partially digested casein) media supplemented with the detergent Tergitol NP40 to decrease the surface tension of the media. *B. cereus* exhibited sliding motility readily on this media. This tryptone and NP40 medium (TNP40) became our standard for observing sliding motility of *B. cereus*.

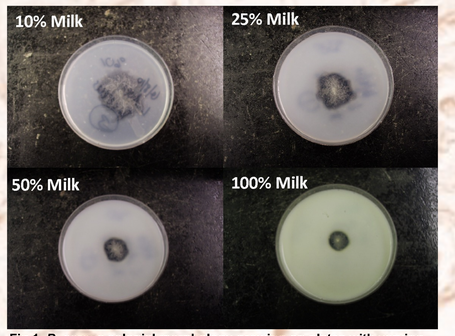

Fig 1. *B. cereus* colonial morphology growing on plates with varying concentrations of milk. *Bacillus cereus* strain 14579 was the standard strain used in these studies. Bacteria were spot inoculated on to milk agar plates using sterile toothpicks and incubated at 30º C for 24 h. Dendritic sliding morphology is evident on all plates.

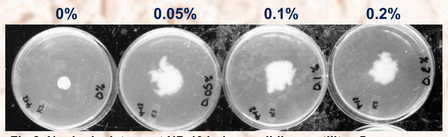

Fig 2. Nonionic detergent NP-40 induces sliding motility. *B. cereus* was inoculated in the center of 3% tryptone agar containing increasing concentrations of NP40. Altered colonial morphology is observed at NP40 concentrations > 0.05% (w/v).

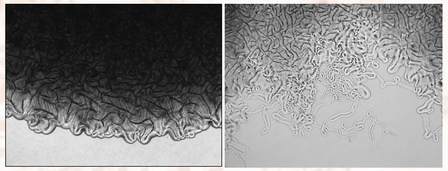

Fig 3. Sliding vs. swarming motility in *B. cereus* colonies. *B. cereus* was spot inoculated on to glass slides coated in a thin layer of agar medium and incubated at 30º C for 22 hours. Colonies were observed by light microscopy at 100X magnification. At left is tryptone media without NP40 and at right is tryptone media with NP40 (0.1%) added.

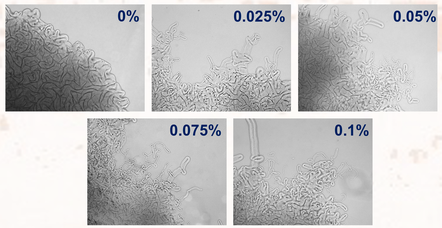

Fig 4. Effect of NP-40 concentration on colonial morphology. Bacteria were spot inoculated on agar slides coated in media containing 3% tryptone and varying concentrations of NP40 and incubated at 30° C for 22 hours. Colonies were observed by light microscopy at 100X magnification. Filamentous outgrowths and "van Gogh bundles" (3) are evident with increasing concentration of detergent. This is consistent with sliding motility.

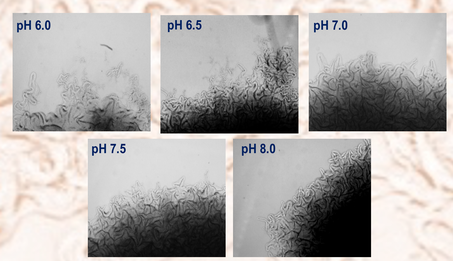

Fig 5. Increasing pH inhibits *B. cereus* sliding motility. Bacteria were spot inoculated on agar slides coated in pH-adjusted media containing 3% tryptone and 0.1% NP40 and incubated at 30° C for 22 h. Colonies were observed by light microscopy at 100X magnification. Sliding motility is reduced at pH > 6.5.

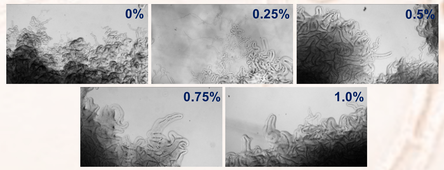

Fig 6. Increasing glucose reduces *B. cereus* sliding motility. Bacteria were spot inoculated on agar slides coated in media containing 3% tryptone, 0.1% NP40, and varying concentrations of glucose. Slides were incubated at 30° C for 22 h. Colonies were observed by light microscopy at 100X magnification. Sliding motility is reduced at glucose conc. > 0.25%.

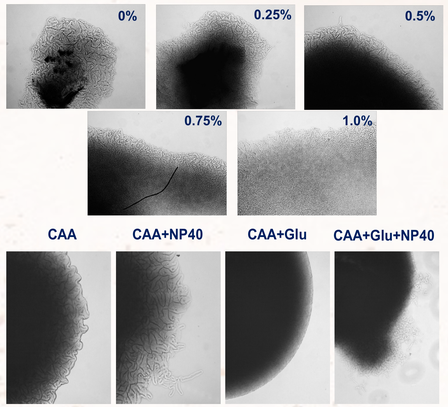

Fig 7. Growth of *B. cereus* on casamino acids + glucose alters colonial morphology. Top: *B. cereus* was spot inoculated on agar-coated slides containing 3% casamino acids and varying concentrations of glucose (0%-1%). Colonies were observed by light microscopy at 100X magnification. **Bottom:** Comparison of morphology between *B. cereus* colonies growing on casamino acid media with and without 0.1% NP40 and 1% glucose added. Colonies were observed by light microscopy at 100X magnification.

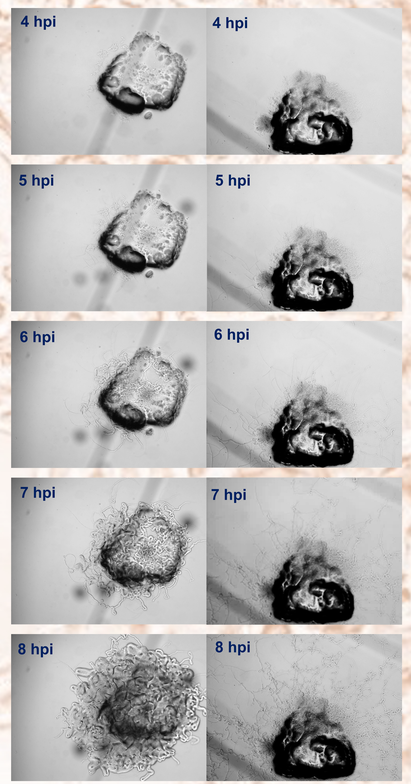

Fig 8. Time-lapse analysis of colony formation 4-8 hours post inoculation (hpi). *B. cereus* was spot inoculated on to 3% tryptone agar coated glass slides and incubated at 30ºC for 4 h. Slides were then incubated and observed on a microscope at 30ºC and photographed at 5 min intervals (100X magnification) for time-lapse videography. Images above are stills at 1 h intervals.

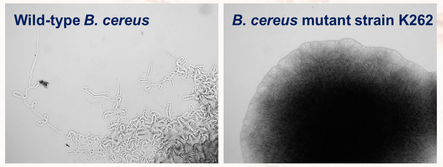

Fig 9. Non-sliding mutant strains of *B. cereus*. *B. cereus* 14579 was transformed by electroporation with plasposon pRL27 (4), and Transconjugants were selected by growth on 3% tryptone agar containing 50 µg/ml kanamycin. Isolates were screened for sliding motility by spot inoculation on 3% tryptone agar containing 0.1% NP40 and 50 µg/ml kanamycin. Non-sliding colonies were then grown on TNP40 agar coated slides for light microscopy at 100X magnification. One of the mutant isolates, K262, is shown on the right, and does not exhibit the morphology associated with sliding.

CONCLUSION

- *B. cereus* sliding motility is stimulated by increasing concentrations of the nonionic detergent, NP-40
- Increasing pH inhibits sliding in *B. cereus*
- Increased glucose concentrations cause a reduction in B. cereus sliding, but does not eliminate it, suggesting that sliding is not purely a result of nutrient starvation
- Addition of glucose to casamino acids agar alters colonial morphology of sliding and non-sliding cells
- Time-lapse photography reveals distinctly different patterns of colony development between *B. cereus* colonies in sliding and non-sliding conditions

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