Adhesion of *Vibrio cholerae* to Granular Starches

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Cholera is a severe diarrheal disease caused by specific serogroups of *Vibrio cholerae* that are pathogenic to humans. Cholera can become epidemic and deadly without adequate medical care. Appropriate rehydration therapy can reduce the mortality rate from as much as 50% of the affected individuals to <1%. Thus, oral rehydration therapy (ORT) is an important measure in the treatment of this disease. To further reduce the symptoms associated with cholera, improvements in oral rehydration solution (ORS) by starch incorporation were suggested. Here, we report that *V. cholerae* adheres to starch granules incorporated in ORS. Adhesion of 98% of the cells was observed within 2 min when cornstarch granules were used. Other starches showed varied adhesion rates, indicating that starch source and composition play an important role in the interaction of *V. cholerae* and starch granules. Sugars metabolized by *V. cholerae* showed a repressive effect on the adhesion process. The possible mechanisms involved are discussed. Comparing *V. cholerae* adhesion with the adhesion of other pathogens suggests the involvement of starch degradation capabilities. This adhesion to granular starch can be used to improve ORT.

Cholera is a severe diarrheal disease that causes the death of many thousands of people each year and affects the lives of millions. The disease is caused by specific serogroups of *Vibrio cholerae* that are pathogenic to humans (21). Since 1991, the world has witnessed the extension of the seventh pandemic into South America and South Africa, as well as the appearance of a previously unknown pathogenic serogroup of *V. cholerae* (O139) (28).

Diarrheal diseases, including cholera, are one of the two main causes of infant and child mortality in developing countries, claiming the lives of >3 million children every year (4). The need to provide immediate assistance to the population at risk led the World Health Organization (WHO) to develop oral rehydration therapy (ORT), enabling the survival of the affected patients through the course of the disease. The life-saving effect of oral rehydration solution (ORS) is achieved primarily by maintaining the electrolyte balance through stimulation of the absorption of sodium from the small intestine (7). The solutions, however, fail to prevent or reduce to a significant extent the symptoms of cholera. In controlled studies, oral rehydration therapy is very effective in reducing mortality (1). Unfortunately, the level of use of oral rehydration therapy has remained low in both developing and developed countries, even though it has been publicized through health education efforts (2, 3, 27). In developing countries, several factors are responsible for the poor acceptance of oral rehydration therapy. There is a perception that oral rehydration is not effective because it does not reduce the manifestations of diarrhea, such as the loss of fluid in feces, or the duration of diarrheal illness (9, 16). Moreover, the glucose-based ORS recommended by the World Health Organization may paradoxically increase fecal fluid loss. Because of these limitations, there has been a substantial impetus to develop a better oral rehydration therapy (33). As ORS is not a prescription drug, it may be used as the frontline treatment of cholera, where timely antibiotic treatment or intravenous rehydration may not be available. This is especially true in regions where medical care is inaccessible within a reasonable time. In these cases, the immediate use of ORS may be lifesaving.

Starch is one of the most abundant carbohydrates in plants. Some of the starch ingested by humans escapes digestion in the small intestine. This fraction was given the name resistant starch (RS). Starch in plants is packaged in granules and can be fractionated into two glucose homopolysaccharide macromolecules: amylose and amylopectin. For most native starches, X-ray diffraction yields two types of spectral patterns, A-type (most cereals) and B-type (tuber) crystallites (15). RS is classified into four types (10, 11). Type I (RSI) is physically inaccessible, because the intact plant structure hinders enzymatic digestion. RSII is a native granular starch with a highly dense crystalline structure that prevents enzymatic digestion. RSIII results from pasted starch formed by a hydrothermal procedure, followed by retrogradation. RSIV is a chemically modified starch. Although not digested in the small intestine, RS may be fermented by the microbiota in the large bowel. The crystalline-resistant structures in RSII and RSIII are resistant to mammalian amylases but can be degraded by microbial fermentation. Among its many beneficial biological effects, RS is known as a source of short-chain fatty acids (SCFA), due to RS fermentation in the colon. Since SCFA are known to en-
hance ion adsorption in the colon, the addition of starch was considered for ORS improvement.

Recently, new endeavors were made to improve ORT by changing the ORS composition. The mechanisms behind the beneficial effects of ORS treatment on human physiology have been studied extensively. However, very little is known about the effects of ORS on the behavior and survival of diarrhea-producing microbes. Beneficial effects of starch-based ORS were proven for the treatment of cholera (26). It is very likely that several mechanisms are responsible for the overall beneficial effects of adding resistant starch to the ORS. Clinical trials with such a mixture showed a marked improvement in symptom manifestation, in addition to the ORS lifesaving effect (26). The hypothesis raised by Ramakrishna et al. (26) was that some of the beneficial effect of the ORS, with its high-amylose cornstarch, was due to SCFA formation by the colon microbiota, thus changing the fluid balance in the colon.

It is known that V. cholerae adheres to different surfaces such as the human lumen, chironomid egg masses (6), chitin (8), zooplankton (20), and other surfaces. For some surfaces, V. cholerae biofilm formation and adherence have been shown to influence its survival and possibly its infective potential (39). The property of V. cholerae to adhere to various surfaces is related to environmental survival of the bacteria (8), as well as to its colonization of the intestine (21, 28, 37). We therefore set out to examine the interactions between V. cholerae and starch. This can explain, at least in part, the beneficial effect of starch-containing ORS in the treatment of cholera compared to the treatment with regular ORS.

MATERIALS AND METHODS

Vibrio cholerae strains and other bacteria used. Vibrio cholerae O1 Inaba was a kind gift from E. Arakawa (National Institute of Infectious Diseases, Tokyo, Japan); V. cholerae O139 was a kind gift from T. Ramarmurthy (National Institute of Cholera and Enteric Diseases, Calcutta, India); and V. cholerae O9 was an isolate from chironomid egg masses (17). Listeria monoyogenes was a kind gift from C. Hill (14). The other strains were from local stock: Aeromonas hydrophila ATCC 33654, Pseudomonas aeruginosa ATCC 27853, Salmonella enterica serovar Typhimurium ATCC 14028, and Escherichia coli ATCC 43895.

Bacterial culturing. All strains, except L. monocytogenes, were grown in 50 ml of rich medium (Luria-Bertani [LB] medium) at 37°C in a 250-ml flask with shaking (200 rpm) for 6 to 8 h. Where appropriate, the culture medium was supplemented with antibiotics at the following concentrations: ampicillin, 100 µg · liter⁻¹; kanamycin, 30 µg · liter⁻¹; and streptomycin, 30 µg · liter⁻¹. L. monocytogenes was cultured in 50 ml of brain heart (BH) medium (32) supplemented with 5 µg · liter⁻¹ erythromycin in a 250-ml flask with shaking (200 rpm) at 30°C for 6 to 8 h.

V. cholerae, as well as the other strains used, was distributed into aliquots containing preservation medium (final glycerol concentration, 20%). The aliquots were kept at −80°C until use. Cultures were reheated from the frozen stock by inoculation of 50 ml of LB or BH medium with the content of one aliquot (and supplementation with antibiotics if used). The cultures were grown for 18 h at 37°C or 30°C with aeration. The cells were harvested by centrifugation (16,000 × g; 5 min).

Plasmid purification and electroporation. E. coli harboring a green fluorescent protein (GFP)-expressing plasmid (pSMC2 or pVPS61TIR) was grown overnight in LB medium containing the appropriate antibiotic. Plasmids were purified with a plasmid purification kit (QiAprep miniprep kit; QIAGEN, Hilden, Germany) and kept at −20°C until use. pSMC2 in E. coli DH5α was a kind gift from R. Kolter (36). pVPS61TIR in E. coli JM109 was a kind gift from S. E. Lindow (23).

Plasmid transformation was done by electroporation as described by Ha-

mashima et al. (18). After electroporation, the cells were grown for 1 h with agitation at 37°C and then streaked on plates with the appropriate antibiotic. Green fluorescent colonies were isolated.

Microscopy. Epifluorescence microscopy was done with a Zeiss Axioscope II equipped with a UV Lamp (Nikon). A filter set with an excitation wavelength of 490 nm and an emission wavelength of 520 nm was used. Digital pictures were taken with a Nikon Coolpix 995 digital camera. Pictures were digitally processed with Photoshop 7.0 (Adobe).

Composition of the ORS. The modified ORS composition was 3.5 g · liter⁻¹ of NaCl, 2.5 g · liter⁻¹ NaHCO₃, and 1.5 g · liter⁻¹ of KCl (WHO formula) (24) without glucose and was used in the standard adhesion experiments. Whenever sugars or salts were added, their final concentration was 111 mM · liter⁻¹ (equivalent to the WHO recommended glucose concentration), as follows: lactose, 38 g · liter⁻¹; sucrose, 38 g · liter⁻¹; trehalose, 38 g · liter⁻¹; glucose, 20 g · liter⁻¹; fructose, 20 g · liter⁻¹; maltose, 38 g · liter⁻¹; xylose, 16.5 g · liter⁻¹; maltodextrins DE19 (for dextrose equivalent 19) and DE6, 50 g · liter⁻¹; and NaCl, 6.5 g · liter⁻¹ (additional). All chemicals were of analytical grade.

Starch degradation activity and starch plates. The starch plate assay employed a solid medium (LB or BH) prepared with 2% (wt/vol) soluble starch from potatoes (Sigma Chemical Co.). Bacteria were streaked on the agar. Plates were incubated at 37°C for 48 h. A solution of 2% (wt/vol) potassium iodide and 0.6125% (wt/vol) iodine was added to the plates to visualize zones of starch hydrolysis.

Starch chemophysical analyses. (i) Size distribution by light scattering. The average granule diameter for each starch type was determined by using a laser diffraction particle size analyzer (LS 230; Coulter). The test was performed following the manufacturer’s instructions. Data were analyzed using the LS Coulter 230 software package, version 2.11a.

(ii) Chemical tests and protein content. The protein contents of each starch type were determined at the Miloda Laboratories, Miloud Industrial Park, Israel (a certified food testing laboratory) according to AOAC International official method 997.06.

Starch preparation. The following types of starch granules were used: corn, wheat, soluble starch from potato, rice (Sigma), high-amylose maize starch, and waxy cornstarch (National Starch & Chemical). All native starches were by definition resistant starch type II. Each starch was equilibrated in the test medium, shaken well, and mixed.

Adhesion procedure. The bacteria, following overnight growth, were harvested by centrifugation and washed three times with 1 ml of the modified ORS. ORS with starch was inoculated with the washed bacterial cells to give an estimated final concentration of 10⁶ cells · ml⁻¹ with 10% starch. After the starch and the cells were mixed for a short period of time (2 min overall time), the starch granules were removed by centrifugal sedimentation (320 × g for 30 s). The number of the remaining bacteria (in the upper fluid) was enumerated by being plated on LB agar or BH agar, with the appropriate antibiotic, if used. All experiments were repeated at least three times with fresh bacteria, starch, and ORS.

The use of controls passing through the same procedure overruled the possibility that the low rate and short centrifugation time were causes for the reduction in CFU recorded. Light microscopy was used to verify the removal of starch granules from the supernatant. The other explanation, attributing the reduction in CFU recorded to the combination of centrifugation and suspended particles, was shown to be irrelevant when particles of soluble starch were used. Those particles did not alter the number of CFU recorded compared with the control.

When slides were prepared for fluorescent microscopy, GFP-tagged bacteria were prepared as described above. In addition, the starch granules were further washed three times after the initial sedimentation with fresh ORS before they were viewed under the fluorescent microscope.

RESULTS

Adherence of V. cholerae O1 to cornstarch granules. V. cholerae O1 cells were grown to stationary phase in LB medium, harvested, washed, and suspended in the modified ORS. The bacterial suspension was mixed with the starch to give a final starch concentration of 10%, and a final ratio of approximately 10⁶ bacterial CFU to 1 g of starch was attained. The mixture was rotated and agitated for 2 min; samples were drawn and spun at 320 × g for 30 s to remove the majority of the starch granules. The supernatant from each sample was serially diluted and plated on LB agar plates, and CFU from the different dilutions were recorded after 24 h. Controls containing bacteria suspended in modified ORS without starch were treated by the same procedure and used to normalize the
were harvested by centrifugation (16,000 g for 5 min) and washed three times with modified ORS without starch. The bacteria were then diluted to a final concentration of \(-10^6\) CFU \cdot ml^{-1}. The starch-containing ORS (preequilibrated) was mixed at a 9:1 ratio to give a final concentration of 10% starch and approximately 10^5 CFU \cdot ml^{-1} (10^6 CFU to 1 g of starch). The mixture was rotated on an axial spinning agitator, and samples were drawn after 2 min. The samples were spun centrifuged for 30 s at 320 × g. A 100-μl sample of the supernatant was serially diluted and plated on Luria agar plates; after 24 h incubation at 37°C, the numbers of CFU were recorded. Controls containing modified ORS without starch were used to normalize the results (P < 0.05). a, b, and c indicate significant differences (P < 0.05).

Starch type

FIG. 1. Immediate adhesion of \textit{V. cholerae} to different starches. \textit{Vibrio cholerae} O1 was grown in 50 ml of Luria-Broth liquid medium in a 250-ml flask with agitation for 16 to 18 h at 37°C. The bacteria were harvested by centrifugation (16,000 g for 5 min) and washed three times with modified ORS without starch. The bacteria were then diluted to a final concentration of \(-10^6\) CFU \cdot ml^{-1}. The starch-containing ORS (preequilibrated) was mixed at a 9:1 ratio to give a final concentration of 10% starch and approximately 10^5 CFU \cdot ml^{-1} (10^6 CFU to 1 g of starch). The mixture was rotated on an axial spinning agitator, and samples were drawn after 2 min. The samples were spun centrifuged for 30 s at 320 × g. A 100-μl sample of the supernatant was serially diluted and plated on Luria agar plates; after 24 h incubation at 37°C, the numbers of CFU were recorded. Controls containing modified ORS without starch were used to normalize the results (P < 0.05). a, b, and c indicate significant differences (P < 0.05).

results. The experiments were repeated at least three times in different days with new ORS, bacteria, and starch. From these experiments, it was evident that as much as 98% of the suspended bacterial CFU compared with the controls were absent from the supernatant after 2 min of incubation with the cornstarch granules (Fig. 1), indicating that \textit{V. cholerae} adheres to the starch granules.

Adherence of \textit{V. cholerae} O1 to other starches. The six different types of starch used, cornstarch, high-amylose cornstarch, waxy maize starch, rice starch, instant starch (soluble starch; potato), and wheat starch, were tested as possible surfaces on which \textit{V. cholerae} O1 could adhere when suspended in the modified ORS. From Fig. 1, it is clear that different starches show diverse interactions with \textit{V. cholerae}. These starches differ in their plant sources, chemical compositions, and other physiochemical properties but are similar in terms of particle size and total surface area (Table 1). In contrast to the marked effect of cornstarch on the number of CFU in \textit{V. cholerae} found in the supernatant of the starch suspension (only 2% unbound CFU), granules of the instant starch did not bind \textit{V. cholerae} in a significant manner. High levels of bacterial adhesion were also detected in suspensions of rice starch, waxy maize starch, and high-amylose cornstarch. Poor adhesion was detected when wheat starch was used. Although distinct, there were no major differences in the particles sizes and total surface areas of the different starches (Table 1). Thus, starch source and composition play an important role in determining the fate of the bacteria (Fig. 1) and not the available surface area or particle size. Vigorous and long agitation in fresh medium detached <10% of the bacterial CFU from the cornstarch granules (data not shown), demonstrating the strength of the adhesion.

Presence of \textit{V. cholerae} O1 cells on cornstarch granules. To locate the bacteria on the granules and to show directly that the bacteria missing from the supernatant are indeed on the starch granules, the same experiments were repeated (including enumeration) with \textit{V. cholerae} O1 that was tagged with a plasmid expressing GFP. Similar numerical results for the adhesion were obtained (data not shown); in parallel to the enumeration of the tagged bacteria left in the upper fluid, the starch granules that were sedimented were washed three times with fresh modified ORS. After the extensive washing, the starch granules were viewed under the fluorescent microscope (Fig. 2). It is evident from Fig. 2 that the \textit{V. cholerae} O1 cells were physically attached to the starch granules. The number of bacterial cells found on each granule varied, and some granules did not have any bacterial cells attached to them. This observation was supported by the fact that the ratio of starch to

### TABLE 1. Chemophysical characteristics of the starches used

<table>
<thead>
<tr>
<th>Property</th>
<th>Corn</th>
<th>Waxy maize</th>
<th>High-amylose corn</th>
<th>Rice</th>
<th>Instant (soluble, potato)</th>
<th>Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Sigma-Aldrich</td>
<td>National Starch &amp; Chemical</td>
<td>National Starch &amp; Chemical</td>
<td>Sigma-Aldrich</td>
<td>Sigma-Aldrich</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>Catalog no.</td>
<td>S4126</td>
<td>00KKH323</td>
<td>HAH333</td>
<td>S7260</td>
<td>S6260</td>
<td>S5127</td>
</tr>
<tr>
<td>Batch no./lot</td>
<td>122K0155</td>
<td>013K0030</td>
<td>013K0030</td>
<td>023K0108</td>
<td>032K1242</td>
<td></td>
</tr>
<tr>
<td>% Protein</td>
<td>0.94</td>
<td>0.35</td>
<td>0.75</td>
<td>0.86</td>
<td>0.21</td>
<td>0.36</td>
</tr>
<tr>
<td>Vol wt mean diam (μm)</td>
<td>13.7, 14.7, 6.397</td>
<td>15.15, 15.31, 8.673</td>
<td>13.96, 13.31, 8.777</td>
<td>8.744, 7.714, 6.281</td>
<td>40.35, 40.24, 17.64</td>
<td>18.07, 18.97, 8.888</td>
</tr>
<tr>
<td>Surface area weighted</td>
<td>5.892, 1.499, 6.781</td>
<td>5.513, 1.426, 7.291</td>
<td>5.626, 1.663, 6.848</td>
<td>3.646, 1.566, 4.311</td>
<td>15.89, 2.527, 19.72</td>
<td>8.734, 3.617, 9.012</td>
</tr>
<tr>
<td>Arithmetic mean diam (μm), D[3,2]</td>
<td>0.728, 0.607, 0.655</td>
<td>0.749, 0.622, 0.626</td>
<td>0.728, 0.598, 0.666</td>
<td>0.745, 0.600, 0.617</td>
<td>0.910, 0.734, 0.962</td>
<td>0.853, 0.636, 0.950</td>
</tr>
<tr>
<td>Viscosity</td>
<td>ND</td>
<td>1.200 BU</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

\[a\] Data shown are mean, median, and standard deviation.

\[b\] ND, not determined; BU, Braebender units.
bacterial cells was kept below the breaking point of the adherence curve (>10^8 bacteria/g of cornstarch) (data not shown).

**The influence of low-molecular-weight solute on the adherence of V. cholerae O1 to cornstarch.** The modified ORS was prepared following the WHO ORS formula without glucose to partially simulate the conditions in vivo, where glucose is readily absorbed in the lumen. Interestingly, adhesion to cornstarch was practically prevented when the unmodified, glucose (111 mmol · liter^-1) containing ORS (Fig. 3) was used instead of the modified ORS. Following these findings, the effect of additional low-molecular-weight carbohydrates on the adhesion to starch was studied. The results obtained with other low-molecular-weight solutes imply that a possible specific adhesion mechanism may be involved. From the sugars tested, those that significantly influenced *V. cholerae* adhesion to cornstarch (Fig. 3) were those utilisable by *V. cholerae* (glucose, maltose, sucrose, trehalose, dextrins [DE6 and DE19], and fructose). The sugars that were not fermented by *V. cholerae* (xylose and lactose) did not prevent the adhesion. This observation points to a possible competition of these carbohydrates with the ligand-binding site or a catabolic repression mechanism involved in the attachment process. The possibility that osmotic pressure is involved was ruled out, as there was no influence of sodium chloride presence (Fig. 3). Moreover, all solutions used had the same osmolarity.

**Adhesion of other pathogenic bacteria to cornstarch.** Examination of the adhesion of other pathogenic bacteria was conducted with strains of *L. monocytogenes*, *S. enterica* serovar Typhimurium, *P. aeruginosa*, *E. coli*, and *A. hydrophila*. A verity of results were observed, ranging from 1% to 57% attachment for *L. monocytogenes* and *A. hydrophila*, respectively (Table 2). The adherence of the other serogroups of *V. cholerae*, *V. cholerae* O139 (which is known to cause pandemics) and O9, was of the same magnitude as that of the O1 strain, with adherence of 83% and 64%, respectively. There seems to be a direct correlation between the adhesion of different bacteria to cornstarch and the ability of the microorganism to utilize this carbon source as determined by starch degradation activity on starch plates (data not shown); *A. hydrophila* and *V. cholerae* are amylase positive and adhere, while the rest of the bacteria tested are amylase negative and show no significant adherence.

**DISCUSSION**

The present study reports for the first time that *V. cholerae* adheres to cornstarch granules. In the presence of cornstarch granules, >98% of the bacteria suspended in the ORS were eliminated from the fluid within 2 min of incubation (Fig. 1). The direct observation of the GFP-tagged bacteria on the cornstarch indicates that the results of the quantitative exper-
iments presented in Fig. 1 are due to an adhesion of *V. cholerae* to the starch granules that was not disturbed by the vigorous agitations during the washing steps; the results could also not be attributed to a change in the number of bacteria that forms a colony on the plates, a change that might have been affected by the presence of starch in the modified ORS. As the fluorescent microscopy of three-dimensional bodies that is available to us hinders a direct count, we can only roughly estimate that most of the bacteria missing from the upper fluid are indeed bound to the starch granules. Also, it should be noted that the direct counts represent a maximal number of CFU; even if some particles were found in the upper fluid, the bacteria attached to those suspended particles were counted as unattached bacteria.

Adhesion experiments with various starches demonstrated that *V. cholerae* adheres to starch granules, to cornstarch in particular. We report marked differences in adhesion of *V. cholerae* to starches from various sources. However, we cannot point to specific compositional characteristics of cornstarch that may be related to the high adhesion to this specific starch. It should be noted that all of the native starches are resistant starch type II (by definition), which is likely to escape digestion in the intestine. Thus, these findings point at a new mode of action, explaining starch function in ORS, and may be utilized to improve the current treatment of cholera.

An interesting question is whether the adhesion is a non-specific physical phenomenon or an interaction between specific ligands. A partial answer can be found in the observation that the adhesion to cornstarch is affected by low-molecular-weight solutes, in particular, sugars. At least two possible explanations can account for these findings. The first explanation attributes the phenomena to metabolic regulation. It is supported by the interesting observation that prevention of adhesion by a solute was related to the ability of the bacteria to utilize the solute as an energy source. It is known that *V. cholerae* reacts to starvation by producing proteins that are translocated to the cell surface (35). Glucose has been identified as a signal that could modulate the expression of virulence factors in *V. cholerae* (31), and a similar mechanism may modulate adhesion to starch granules. Further support for the relation between starch metabolism and the adhesion can be found in the adhesion of other pathogen strains. The adhesion to starch, among the small panel of pathogenic bacterial strains, seems to be unique to those that are able to utilize it, at least as judged by the screen performed in this study.

The second possible explanation for the repressive role of several sugars on the adhesion is that of competition between the starch and the solute for specific binding sites found on the bacteria. It is well documented that pathogenic bacteria, including *V. cholerae*, express lectins on their surface to adhere to the host tissues (29). In addition, *V. cholerae* expresses a malto-dextrin (a product of the degradation of starch)-binding protein that was recently cloned and characterized (13). Of particular interest is the mannose- and glucose-specific lectin isolated from the O1 strain by Sasmal et al. (30). Thus, it is possible that the adhesion-inhibitory effect of some of the sugars tested is due to their competitive binding to lectin-binding sites involved in the adhesion to starch granules. In this context, the fact that maltose was shown to affect the virulence of *V. cholerae* (22) may indicate an additional benefit of starch, namely, virulence repression by an available carbon source.

The adhesion to starch described here is an interesting feature of *V. cholerae* as it might be related to its survival in the environment during and between epidemics. Starch is an abundant natural polymer. The ability to utilize starch, as well as other complex polymers, like the sugar-containing gelatinous matrix surrounding the chironomid egg masses (17), indicates that this microorganism relies on several complex substrates for its survival. It has been speculated that the appearance of high numbers of *V. cholerae* is correlated to algal blooms (25), yet algae are known to contain large quantities of starch. In addition, *V. cholerae* could be isolated from plants (Eichhornia crassipes) found in river water (5). Therefore, the ability to utilize starch as well as the ability to adhere to it can contribute to the utilization of this food source in the aquatic environments.

The results presented here are of high importance, as they may explain several unresolved issues in the matter of the beneficial effects attributed to starch incorporation into ORS. It is well documented that starch incorporation into ORS does not benefit all cases of diarrhea. In fact, the beneficial effects of starch on ORT were shown only when the disease was caused by *V. cholerae*. Even in the case of starch incorporation in ORS treatment of cholera, a wide range of results were obtained, ranging from no effect to marked improvement in symptoms and reduction in the duration of the disease (19, 26, 38). Our findings shed light on these puzzling results and may explain, at least partially, the confusing data. We hypothesize that during diarrhea, positive effects such as SCFA formation, attributed to the regular consumption of resistant starch, disappear. These well-documented effects are intimately linked to the natural microflora of the gut (34), which is washed out during the severe fluid loss. Thus, beneficial effects from starch, when incorporated into ORS, are either related to its physical properties and its interaction with the human body or due to the diarrhea-causing-agent interactions with the starch. As starch incorporation seems to be beneficial in a manner related to the causative agent (12), we claim that the latter prevails. When no interaction exists between the causative agent and the starch, starch incorporation into ORS has no significant effect. In contrast, *V. cholerae* interacts with starch, resulting in a marked effect on the ORT. In accordance with our findings, we suggest that adhesion to the starch granules might compete with sites in the lumen during colonization. We suggest a model where *V. cholerae* adheres to the ungelatinized starch granules, which are practically indigestible in the intestine and are secreted from the intestine. It is therefore possible that in this way that
the adhering *V. cholerae* is removed from the intestine. Yet, as is evident from our results, starch type also plays a pivotal role in the strength of the interaction. In addition, the microflora found in the gut most probably utilizes the glucose found in the ORS. As showed in this study, the presence of glucose prevents the adhesion of *V. cholerae* to the starch granules. These observations imply that starch type incorporated into the ORS, as well as the ORS composition, should be chosen carefully.

The points addressed in this article can partially explain the wide range of results obtained in the clinical trials in different starch-containing ORS formulas. Further studies are under way to identify the nature of the adherence factors, their regulation, and their possible effects on cholera toxin expression.

REFERENCES