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# MICROBIAL EXAMINATION OF SOLAR, ROCK, AND GRANULATED SALTS AND THE EFFECT OF THESE SALTS ON THE GROWTH OF CERTAIN SPECIES OF LACTIC ACID BACTERIA[1]

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## Summary

Samples of solar, granulated, and rock salts (NaCl) were compared as to microbial content and their effect on the growth of four species of lactic acid bacteria. No significant differences in microbial populations were observed in tests of the three types of salt for aerobic bacteria, aerobic spore-forming bacteria, anaerobic bacteria, anaerobic spore-forming bacteria, lactic acid bacteria, coliform bacteria, yeasts, and molds. The four species of lactic bacteria employed grew as well in the presence of solar salt as with rock or granulated salt. The pattern of inhibition with the three types of salts at increasing salt concentrations (5%, 7.5%, and 10%) was typical for the species of lactic acid bacteria tested. A survey for true halophilic bacteria failed to show any marked growth by these microorganisms from either solar salt or the other types of salt investigated. From the data obtained here, it is concluded that solar salt is not likely to be a source of microbial contamination or cause problems if used in the preparation of brine for the fermentation of cucumbers or other vegetables.

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## Introduction

The pickle industry has long used rock salt or granulated salt for brining cucumbers. This study was undertaken to determine whether solar salt might have a deleterious effect on the fermentation of brined cucumbers. Cucumber briners were concerned that solar salt might serve as a source of undesirable bacteria or that it might contain substances which would prevent growth of bacteria responsible for the acid fermentation. Solar salt has been reported to contain certain bacteria that caused undesirable changes in food products such as salted fish (Gibbons, 1936). Warnings about the use of solar salt in other applications have been expressed recently by Levin and Vaughn (1966) and by Michaud and VanDemark (1967).

One group of bacteria, the obligate halophiles, are dependent for growth on high concentrations of salt (12% to saturation). Thus, it appeared important to test for the incidence of these bacteria in solar salt. In addition, this study attempted to answer the following questions: (1) Does solar salt contain appreciable numbers of other bacteria of sanitary significance (coliform bacteria) or of microbes which might affect the fermentation or preservation of the brined material? (2) What is the total population of microbes carried by solar salt? (3) Does solar salt contain substances which might inhibit the growth of the lactic acid bacteria responsible for normal fermentation of commercially brined cucumbers? (4) How does solar salt compare to rock salt and granular salt in these respects? The experiments described below were designed to obtain useful information on these questions.

## MATERIALS AND METHODS

**Sources of salt.** Three different types of salt were used; solar, rock, and granulated. Sixteen salt samples were obtained as described in Table 1. They were collected in sanitized, 6-oz. jars, sealed with "twist-off" caps (White Cap Company, Chicago, Illinois), shipped to Manhattan, Kansas, and held at room temperature until examined. Chemical analyses of typical samples of the three kinds of salt were kindly provided us by Watkins Salt Company, Watkins Glen, New York.

**Cultural procedure.** Population estimates were determined for several groups of microorganisms occurring in the salt samples by the use of selective media and by employing plating, streaking, and growth in liquid media techniques, most of which have been described in detail by other workers. Decimal dilutions of the salt samples were examined for eight microbial groups as follows:

(1) **Total aerobes** were determined by streaking with a calibrated loop onto previously poured plates of glycerol asparaginate agar (Conn, 1921). (2) **Aerobic spores** present in heated samples (80°C for 10 minutes) were enumerated by plating with nutrient agar (Difco). (3) **Total anaerobes** were estimated by plating in the basal yeast extract starch bicarbonate (YESB) agar of Wynne, Schmeiding, and Daye (1955); the plates were incubated in Brewer anaerobic jars or in enclosed vessels with alkaline pyrogallol. (4) **Anaerobic spores** present in heated samples (80°C for 10 minutes) were cultured as described for total anaerobes. (5) **Coliform bacteria** were determined by plating on violet red bile agar (Difco). (6) **Acid-forming bacteria** were estimated by plating on **Lactobacillus** selective medium (BBL), but modified as to the critical pH level (5.6 ± 0.05) for good development of different species of these organisms (Costilow **et al.**, 1964). (7) **Yeasts** and (8) **molds** (filamentous fungi) were determined by streaking onto plates of nitrogen base agar pre-

TABLE 1
Source of salt samples

| Sample no. | Type of salt | Company and location [1] | Description of source [2] of sample at point collected |
|---|---|---|---|
| 1 | Solar | CSC [1] | Arrived Jan. 25, from Tunisia; sample from bottom of face of 3,000 ton pile. |
| 2 | Solar | CSC | As no. 1; about 10-12 feet up face of pile. |
| 3 | Solar | CSC | As no. 1; about 20 feet up face of pile, 3-4 feet below peak. |
| 4 | Solar | CSC | Kiln dried at 160°F for 20 min.; sample taken from enclosed metal storage hopper of 48-ton capacity. |
| 5 | Solar | CSC | As no. 4. |
| 6 | Solar | CSC | From 100 lb bag; salt kiln dried 220-250°F for 20 min.; salt arrived on Oct. 18 or 19, and dried and bagged mid-Jan. |
| 7 | Solar | CSC | As no. 6, but from a different bag. |
| 8 | Rock, C-grade | MOPICO [1] | Stored in open vats; put in vats from late Dec., until Jan. 10, supplied by Cargill; sample from storage tank no. 23-10. |
| 9 | Rock, C-grade | MOPICO | As no. 8, but sample from storage tank no. 22-6. |
| 10 | Rock C-grade | MOPICO | As no. 8, but sample from storage tank no. 21-24. |
| 11 | Rock, C-grade | L & S [1] | Stored in salt house; received first week in Jan.; supplied by International Salt Company. |
| 12 | Rock, C-grade | L & S | As no. 11. |
| 13 | Rock, C-grade | L & S | As no. 11. |
| 14 | Granulated, bulk | PPP [1] | Stored in salt house; probably received in Jan.; supplied by Watkins Salt Company from underground source. |
| 15 | Granulated, bulk | PPP | As no. 14. |
| 16 | Granulated, bulk | PPP | As no. 14. |

[1] CSC = Carolina Salt Company, Wilmington, N. C.; MOPICO = Mt. Olive Pickle Company, Mt. Olive, N. C.; L & S = Lutz and Schramm, Inc., Division of Beatrice Foods Company, Ayden, N. C.; PPP = Perfect Packed Products Company, Inc., Henderson, N. C.

[2] Salt samples were collected February 4 and 5, 1966.

pared from Wickerham's (1951) yeast nitrogen base broth (Difco) as described by Etchells **et al.** (1953, 1958, 1961). This medium was further modified by the addition of 0.01 percent yeast extract and acidification with 1.6 ml of sterile 5.0 percent tartaric acid per 100 ml of melted agar. This medium (pH 3.5) inhibited the bacteria, yet gave good growth of yeasts together with adequate but restricted colonial development of molds.

**Growth of lactics in salt.** The ability of four species of lactic acid bacteria to grow in various concentrations of the three types of salt was tested as follows: A 20 percent (w/v) salt solution of each sample to be tested was sterilized by filtration and diluted with appropriate amounts of a double strength TYE basal broth (tryptone, 2%; yeast extract, 1%; glucose, 2%; $K_2HPO_4$, 0.6%; at pH 6.8 to 7.0) or distilled water to prepare concentrations of 0, 5, 7.5, and 10 percent NaCl. Twelve tubes of each concentration of each salt sample tested were prepared. One drop of a 24-hour broth culture of each lactic species tested (**Lactobacillus brevis,** LB-50; **L. plantarum,** LP-442; **Pediococcus cerevisiae,** PC-39; and, **Leuconostoc mesenteroides,** LM-42) was used to inoculate triplicate series of each sample concentration. All cultures were from the collection of U.S. Food Fermentation Laboratory. The first species named was originally isolated from cucumber fermentations in North Carolina (Borg, Etchells, and Bell, Bacteriol. Proc., p. 19, 1955); the remaining three species were isolated from fermentations in Michigan (Costilow **et al.**, 1956). After incubation, the relative amount of growth was measured in terms of percent light transmittance using a colorimeter (Bausch and Lomb, Spectronic 20) set at 525 millimicrons.

**Examination for halophilic bacteria.** Fifty ml of single strength TYE broth, described earlier, was added to weighed flasks and sterilized. The flasks were reweighed and salt added aseptically to give final concentrations of 0, 5, 7.5, 10, and 15 percent. Salt samples tested included nos. 1, 2, 3, 5, 7 (solar), 9, 12 (rock), and 15 (granulated). Preparations designed to reveal the presence of true obligate halophilic bacteria (at 12% NaCl and above) were examined microscopically and by phase contrast microscopy after two weeks' and six months' incubation at room temperature.

## Discussion of Results

**Chemical analysis of salt.** The chemical analysis of each salt (Table 2) indicated no substances which might inhibit the growth of lactic acid bacteria. Calcium sulfate was found in all three types of salt. Only solar salt had an appreciable amount of magnesium salts (0.053% $MgSO_4$ and 0.203% $MgCl_2$), and rock salt was high in "insoluble materials" (silica?) reaching a level of 0.738%. The high magnesium salt content of solar salt may be responsible for its high moisture content (2%) and may result in an undesirable caking action. The levels of calcium and magnesium salts shown for solar salt would not interfere with the natural fermentation of cucumbers (Bell and Etchells, **unpublished data**).

**Populations of microorganisms in salt.** The bacteriological tests that were made to determine the populations of eight different microbial groups in the sixteen salt samples showed them either to be absent or present in very low numbers (see Table 3). The numbers of each microbial group were very nearly the same in all samples of salt examined, with the exception of the granulated salt samples, which were essentially free of microorganisms. Small numbers of aerobes and anaerobes, largely spore-forming types, were found in low populations (0 to 220 organisms per gram of salt) for the solar and rock salt samples. Coliform and lactic acid bacteria, yeasts, and molds were virtually absent in all the salt samples plated. In considering the data in Table 3, it must be understood that the plate count method suffers from errors of random sampling when the colony count is less than 30 per plate. For this reason, one must be cautious about attributing significance to minor differences in the counts. In fact, the differences between individual samples and between sources of salt are so small that they have no statistical validity in judging the absolute populations of microbes in the samples and are considered only "estimates" for this study. As so aptly put by de Figueiredo (1970), "The sensitivity of the methods used should realistically reflect the purpose intended."

The problem can be illustrated by the data for total aerobes and total anaerobes in rock salt sample nos. 11 and 12 (Table 3). The counts obtained from these two samples of rock salt were the highest of any of the sixteen samples tested. Yet, salt sample no. 13, from precisely the same source, shows very low counts and is characteristic of samples of salt of this and other kinds obtained elsewhere. Such variations are not unexpected with the technique used. The data suggest that most of the microorganisms appearing in the culture plates were probably aerobic spore-forming bacteria. These microbes are abundant in soil and on airborne particles which settle as dust. It is possible that they were introduced through the normal handling procedures and were not part of the indigenous flora of the salt. The microbial population, although very low in unrefined solar and rock salts, which are handled in bulk, were consistently higher than those in refined granulated salt where essentially no microbes were detected (Table 3).

Within the limitations mentioned, the counts obtained for solar salt are slightly lower but of the same order of magnitude as those reported by Michaud and VanDemark (1967). For example, they report total bacterial populations ranging from less than 1 to 590 per gram of solar salt. Compare this to a total count of 80 aerobic and 10 anaerobic colonies per gram of salt in sample no. 2 (Table 3). Again, such comparisons are risky when the counts are so small. Plate counts for rock or granulated salt were essentially the same as those reported by Michaud and VanDemark (1967). There was no real difference between the microbial population of the untreated samples (nos. 1, 2, 3) and kiln dried samples (nos. 4, 5, 6, 7) of solar salt.

**Growth of lactic acid bacteria in the presence of three types of salt.** Four species of lactic acid bacteria (**Lactobacillus plantarum, Pediococcus cerevisiae, L. brevis,** and, to a lesser extent, **Leuconostoc mesenteroides**) are usually considered the ones most frequently found in the natural fermentation of brined cucumbers (Costilow **et al.**, 1956). The first two listed are non-gas-forming species and are highly desirable (Etchells **et al.**, 1964). The growth of these two species at 0%, 5%, 7.5%, and 10% salt concentrations (Figure 1) show no essential difference in growth response among the salt samples tested, whether the origin of the salt was rock, solar, or granulated. The small growth variations at two days' incubation between the salt types, at the three concentrations, follow expected growth patterns for these two species (**L. plantarum** and **Ped. cerevisiae**) and are, in general, in agreement with 48 hour salt-tolerance tests reported by Etchells and Jones (1946) and Etchells et al. (1964).

The two gas-forming lactic acid species (**L. brevis** and **Leuc. mesenteroides**) were also tested for growth in solar, rock, and granulated salt, but the results are not shown graphically because of the rather slow growth in salt brines above 5% for the first species listed, and the known low salt-tolerance of the second species. These species grew in the 5% concentration for each type of salt. The amount of growth at two days was about onethird to one-half that found in the no salt controls. At the higher salt concentrations (7.5% and 10%) there was little or no growth by either species in any of the salt samples tested. The results for these species after a two-day incubation period were typical of their salt tolerances and in agreement with Costilow **et al.** (1956), Davis (1955), Etchells **et al.** (1964), and Etchells and Bell (**unpublished data**). In summary, the growth patterns at two days for the four species of lactic acid bacteria, when tested at three concentrations of solar, rock, and granulated salt, were all considered typical and within expected growth variations of the species.

The tests just described were run specifically to determine if the four lactic species would grow in the presence of the various salts under investigation at concentrations of about 5%, which compares favorably to the equilibrated brine strength now used for cucumbers. For more information on the salt-tolerance of three of the four species of lactic acid bacteria used herein, refer to Table 4.

**Presence of true halophilic bacteria.** When salt was added to the culture medium in such a fashion that it was the source of inoculum for microorganisms, a very limited microbial flora developed. Relatively large populations of bacteria (principally spore-formers) appeared at salt concentrations of 5% and 7.5%. However, at 10% and 15% salt, the flora was much more restricted. At the 10% concentration, spore-forming rods grew but not heavily. At 15%, one of the samples tested showed slight growth of a mold after two weeks' incubation while those from the other seven salt samples were negative. Over a period of about six months a slight to heavy mold growth developed in cultures from all samples except no. 15. Bacteria were seen at six months but not at two weeks in samples 3, 5, 7, and 12. It was difficult to tell whether the spheres seen in samples 5 and 7 were large cocci or structures separated from the mold which was present. These tests were in the nature of a general survey and were not intended as an exhaustive search for halophilic bacteria.

Those who have written about microbes growing in salt brines have contributed a good deal of confusing terminology. One often encounters terms such as "salt tolerant" and "halophilic" used interchangeably and without specification of the minimum salt concentration at which the organisms must grow in order to qualify under the name. Thus, it is difficult to interpret the data presented by Michaud and VanDemark (1967) in terms of the actual tolerance of "salt tolerant" bacteria, especially in relation to "halophiles." The true, obligate halophilic bacteria found in untreated solar salt require a salt concentration of **at least** 12% and will grow in saturated salt brines (Elazari-Volcani, 1940). Halophilic strains of other bacteria such as **Aerobacter** (Etchells and Jones, 1943) have been obtained from cucumber brines, especially in salt concentrations in the range of 12 to 15%. The approach used by Levin and Vaughn (1966) was to place the sulfate reducing bacteria that they studied in ". . . two general groups: 1) optimum growth without NaCl . . .; and 2) obligately halophilic growth, requiring at least 0.5% NaCl . . ." In this muddled situation, any discussion of halophiles must be preceded by agreement on definition. For solar salt the classical definition used by Elazari-Volcani (1940) should be invoked.

The evidence presented here and the references cited in this report suggest that solar salt could be used without deleterious effect in brining food products that undergo an acid fermentation. There are two main reasons for this statement. First, special groups of microbes which conceivably might influence the fermentation or which indicate unsanitary conditions, are virtually absent from solar salt as they are from mined salt. Second, growth of the true halophilic bacteria is strongly inhibited in acid conditions; these organisms fail to grow at all below pH 5.5. This brine pH level is usually reached in 48 hours or less in the natural fermentation of brined cucumbers (Jones, 1940) and, thus, the growth of halophiles from solar salt would be completely prevented; assuming, of course, these organisms could even initiate growth at the starting brine strengths now generally in use by the pickle industry (5-7% by weight; Etchells and Moore, 1971).

Warnings about possible dangers in the use of solar salt in preserving foodstuffs are apparently aimed at products which are near neutral in pH; for example, salted fish (Gibbons, 1936). Indeed, the classical solar salt halophile, **Halobacterium cutirubrum,** was found to be extremely sensitive to acid conditions (Kushner and Bayley, 1963) and recently it has been suggested that the organism could be eliminated from solar salt by acidifying the saturated brine from which it is crystallized (Kushner **et al.**, 1965)

In addition, there is good reason to believe that growth of halophiles characteristically found in small numbers in solar salt would have no deleterious effect on fermented products. These halophiles oxidize sugar rather than ferment it and thus, would not contribute to "bloater" spoilage (hollow cucumbers) or other problems resulting from gas production by microbes. They are not highly diverse in their metabolic potential and are not known to produce enzymes that might deteriorate vegetable tissue. Thus, even in the highly unlikely event of growth by solar salt halophiles, the fermentation of brined vegetable products would almost surely be unaffected.

**TABLE 2**
**Typical chemical analysis of granulated, solar, and rock salt.**

| Analysis for | Granulated salt | Tunisia solar salt[1] | Louisiana, grade C, rock salt |
|---|---|---|---|
| | % | % | % |
| Sodium chloride | 99.850 | 99.528 | 99.049 |
| Calcium sulfate | 0.135 | 0.163 | 0.175 |
| Calcium chloride | 0.012 | 0.000 | 0.008 |
| Sodium sulfate | 0.000 | 0.000 | . . . |
| Magnesium sulfate | . . . | 0.053 | . . . |
| Magnesium chloride | 0.003 | 0.203 | 0.011 |
| Insoluble materials | 0.000 | 0.053 | 0.738 |
| Moisture | 0.020 | . . . | 0.019 |

[1] Analysis on dry weight basis; moisture content 2.0%.

TABLE 3

Estimates of the populations of bacteria, yeasts, and molds in salt samples

| MICROBIAL GROUP | MICROORGANISMS PER GRAM FOR SAMPLE NOS. 1-16 | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Solar | | | | | | | Rock | | | | | | Granulated | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Total aerobes | 70 | 80 | 60 | 30 | 20 | 20 | <10 | 0 | 30 | 0 | 30 | 110 | 0 | 0 | <10 | 0 |
| Aerobic spore formers | 0[1] | 20 | 30 | <10 | <10 | 0 | <10 | <10 | 0 | 0 | 50 | 120 | <10 | 0 | 0 | 0 |
| Total anaerobes | 10 | 10 | 10 | <10 | 0 | 0 | 20 | 0 | 0 | <10 | 220 | 60 | 30 | 0 | <10 | 0 |
| Anaerobic spore formers | 10 | <10 | 10 | <10 | 0 | 0 | <10 | 0 | 10 | 0 | <10 | 60 | 0 | 0 | 0 | 0 |
| Coliform bacteria | 0 | 0 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lactic acid bacteria | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <10 | 0 | 0 | 0 |
| Yeasts | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Molds | 0 | <10 | <10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | <10 | 0 | 0 | 0 | <10 | 0 |

[1] Zeros in columns mean a count of < 1 per gram.

TABLE 4

Influence of salt on the growth of three species of lactic acid bacteria in pasteurized cucumbers[1]

| Species | Salt concentration[3] | Maximal plate count: Millions per ml | Maximal plate count: Age in days when reached |
|---|---|---|---|
| | % | | |
| Pediococcus cerevisiae, FBB-61 | 0. | 1,420 | 2 |
| | 4.2 | 1,430 | 2 |
| | 6.3 | 930 | 2 |
| | 8.1 | 620 | 4 |
| | 10.2 | <1 | 25 |
| Lactobacillus plantarum, FBB-67 | 0 | 2,200 | 4 |
| | 4.2 | 910 | 4 |
| | 7.2 | 600 | 4 |
| | 8.3 | 80 | 4 |
| | 10.3 | 6 | 20 |
| Lactobacillus brevis, FBB-70 and L-544[2] | 0 | 905 | 2 |
| | 2.1 | 850 | 2 |
| | 4.2 | 470 | 2 |
| | 6.2 | 293 | 6 |
| | 8.3 | 29 | 6 |
| | 10.3 | <1 | 25 |

[1] Tabulated from data of Etchells et al. (1964), and Etchells et al. (1968).

[2] Average counts for two strains.

[3] Reagent-grade sodium chloride used.


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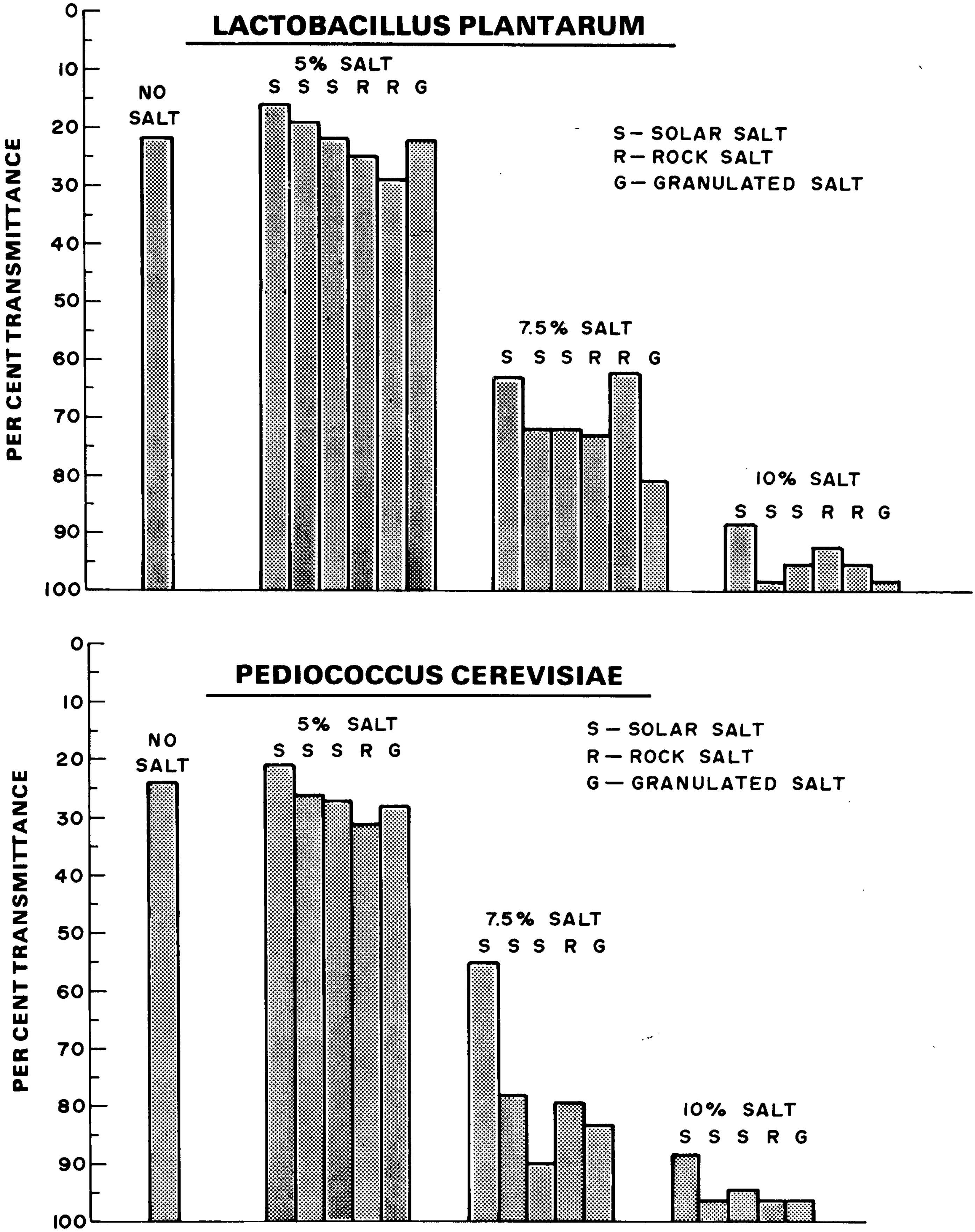


FIGURE 1

*Typical growth patterns of two species of lactic acid bacteria after two days' incubation at 90°F in enriched solar, rock, and granulated salt samples.*