

Inhibitory and Stimulatory Effects of Oregano on *Lactobacillus Plantarum* and *Pediococcus Cerevisiae*

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ABSTRACT

Increasing concentrations of oregano from 0.5 to 8 g/L in a liquid medium resulted in stimulation, delay, or inhibition of acid production and viability of *Lactobacillus plantarum* and *Pediococcus cerevisiae*. The inhibitory factor could be removed from oregano by solvent extraction or autoclaving; residues from these treatments stimulated acid production by the organisms. Organisms growing in media containing sublethal oregano concentrations that had delayed growth and acid production developed resistance to the deleterious effects of the spice. *L. plantarum* was more resistant than *P. cerevisiae* to the inhibitory effects of oregano.

INTRODUCTION

ALTHOUGH SPICES are used primarily for their desirable flavor and odor, they may play other important roles in a food system. Antimicrobial properties of spices (Corran and Edgar, 1933; Fabian et al., 1939; Webb and Tanner, 1945; Dold and Knapp, 1948; Wright et al., 1954; Maruzzella and Freundlich, 1959) and of their essential oils (Collier and Nitta, 1930; Blum and Fabian, 1943; Maruzzella and Henry, 1958; Maruzzella and Ligouri, 1958; Koedam, 1977) have been documented. We have observed that spices stimulate the production of acid by lactic acid starter cultures both in fermented sausages (Zaika et al., 1978) and in a liquid medium (Kissinger and Zaika, 1978; Zaika and Kissinger, 1979a, b). All of the 19 spices tested in a liquid medium stimulated acid production by a commercial starter culture Lactacel MC, composed of *Lactobacillus plantarum* and *Pediococcus cerevisiae*, although at high concentrations a number of the spices were inhibitory. Oregano was the most inhibitory spice tested. Previously, Julseth and Deibel (1974) reported that the growth of *Salmonella* inoculated into pre-enrichment media containing oregano was definitely inhibited. Beuchat (1976) reported that oregano was highly toxic to *Vibrio parahaemolyticus* when present in growth media at a concentration of 0.5%.

We found that although oregano can be bactericidal toward lactic acid bacteria, these organisms can become resistant toward the toxic effect of oregano. This paper describes our studies on the effect of oregano on the viability and acid production of commercial starter cultures of *L. plantarum* and *P. cerevisiae* and the inhibitory and the stimulatory factors involved.

MATERIALS & METHODS

Starter cultures

L. plantarum (Lactacel DS, Microlife Technics, Sarasota, FL), *P. cerevisiae* (Lactacel, Microlife Technics) and a mixed culture of *L. plantarum* and *P. cerevisiae* (Lactacel MC, Microlife Technics) were used for fermentation.

Oregano

Sterilized oregano (Griffith Laboratories, Inc., Union, NJ) was used through the experiment.

Liquid medium

Beef extract (Difco Labs, Detroit, MI), 3g; tryptone (Difco), 5g; sucrose, 20g; and glucose, 20g, were dissolved in 1L of distilled water. The pH of the solution was adjusted to 6.5 with 1N HCl to give a post-sterilization pH of 5.98–6.17. Aliquots of 250 ml of the medium were dispensed into 500 ml Erlenmeyer flasks and sterilized for 15 min at 15 psi.

Fermentation

Oregano was added aseptically to the flasks of sterile media to provide concentrations of 0.5, 1, 2, 3, 4, or 8 g/L. A commercial starter culture, 2.5 ml, diluted with 0.1% peptone water was then added to each flask and to a control without oregano to give an initial bacterial population in the range 10^4 – 10^5 cells/ml. The flasks were incubated statically for up to 7 days at 35°C. Samples for bacterial counts and titratable acidity were taken at 24 hr intervals.

For some experiments, oregano was autoclaved in suspension with liquid medium as described above.

Bacterial counts

Bacterial counts were made by conventional pour plate techniques with APT agar (Difco). Plates were incubated for 48 hr at 35°C.

Titratable acidity

Titratable acidity was expressed as ml of 0.1N NaOH required to titrate to pH 7.0 a 10 ml aliquot of the centrifuged liquid medium after dilution with 50 ml distilled water. The initial titratable acidities of the liquid media were 0.35–0.55 ml.

Extraction of oregano with solvents

Oregano, 2g, was weighed into 33 x 94 mm cellulose extraction thimbles (Whatman) and extracted for 6 hr in a Soxhlet extractor with 130 ml of petroleum ether (b.p. 35–60°C), chloroform, or methanol. After extraction, the thimbles containing the insoluble oregano residues were placed into sterile wide-mouth bottles, capped with cotton, and the solvent was allowed to evaporate. The con-

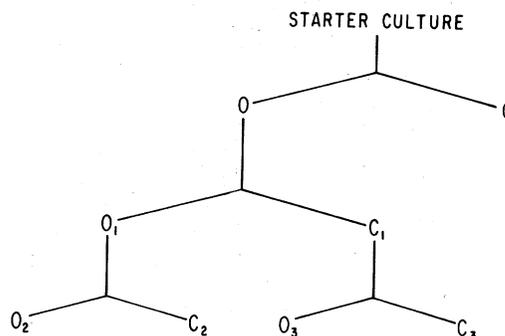


Fig. 1—Sequential subculturing of starter culture bacteria exposed to oregano. O, O₁, O₂, O₃ = medium containing 3 g/L oregano; C, C₁, C₂, C₃ = control medium.

tents of the extraction thimble were added to a flask containing 250 ml sterile medium. The mixture, representing 8 g/L whole oregano, was inoculated and incubated as described above.

Aqueous extract of oregano

Oregano, 2 g, was added to 250 ml sterile water, and the mixture was kept at 5°C for 3 days. The insoluble material was removed by filtration through a Millipore filter, 0.47 μ . The filtrate, 125 ml, was added to 125 ml of sterile double strength liquid medium, and the resulting solution equivalent to 4 g/L oregano was inoculated with *L. plantarum* and incubated as described above. A sample containing 4 g/L whole oregano and a control were also prepared with double strength liquid medium diluted with water.

Oregano residue

Oregano, 10g, was successively extracted at room temperature with chloroform, 2 x 100 ml, ethanol, 4 x 50 ml, and water, 3 x 200 ml. The insoluble residue, collected on a Büchner funnel, was washed with 100 ml ethanol and allowed to dry. Before being added to media inoculated with *L. plantarum*, the residue was sterilized with ethylene oxide in a Cryotherm Portable Sterilizer, Series 8040 (American Sterilizer Co., Erie, PA).

RESULTS & DISCUSSION

THE RESPONSE of *L. plantarum* to various concentrations of oregano is shown in Table 1. In the presence of 0.5 g/L oregano bacterial growth was the same as in a control medium, while the final titratable acidity was 2.5 times as large. Increasing concentrations of oregano progressively delayed bacterial growth and acid production. In the presence of 4 g/L oregano, bacterial numbers at first declined from an initial value of 2.2×10^4 cells/ml to 63 cells/ml, but increased to 6.0×10^6 cells/ml after 5 days. At this time, acid production began and after 7 days was > 2.5 times that in the control medium. A concentration of 8 g/L oregano was bactericidal to *L. plantarum*.

The effect of various concentrations of oregano on *P. cerevisiae* is shown in Table 2. This organism was more sensitive than *L. plantarum* to oregano. *P. cerevisiae* also produced less acid in the presence of oregano than did *L. plantarum*, although in control media acid production was comparable. A concentration of 4 g/L oregano was sufficient to completely inhibit growth of and acid production by *P. cerevisiae*. In the presence of 0.5 g/L oregano, bacterial counts were in the range of the control values, while

Table 1—Effect of oregano on growth of and acid production by *L. plantarum*

Day		Oregano, g/L					
		0	0.5	1	2	4	8
1	TA ^a	0.46	0.85	0.00	0.00	0.00	0.00
	C ^b	1.9×10^8	2.8×10^8	1.9×10^7	1.7×10^3	1.4×10^2	3.0×10^2
2	TA	1.08	2.45	2.50	0.08	0.05	0.03
	C	8.6×10^7	2.9×10^8	2.1×10^8	1.1×10^4	6.3×10^1	$<10^1$
3	TA	1.42	3.34	3.73	2.47	0.03	0.00
	C	5.3×10^7	2.4×10^8	2.5×10^8	7.7×10^7	2.7×10^3	$<10^1$
4	TA	1.67	3.90	4.33	4.24	0.05	0.09
	C	3.2×10^7	5.9×10^7	8.2×10^7	3.7×10^7	2.8×10^4	$<10^1$
5	TA	1.85	4.37	4.62	5.41	0.32	0.10
	C	1.5×10^7	1.8×10^7	2.9×10^7	2.3×10^7	6.0×10^6	$<10^1$
6	TA	1.99	4.75	4.77	6.00	4.40	0.15
	C	9.0×10^6	1.1×10^7	1.1×10^7	1.2×10^7	1.7×10^8	$<10^1$
7	TA	2.00	5.00	4.81	6.13	5.32	0.10
	C	—	—	—	—	—	$<10^1$

^a TA = Titratable acidity produced, ml.

^b C = Bacterial count/ml. The initial bacterial count was 2.2×10^4 cells/ml.

Table 2—Effect of oregano on growth of and acid production by *P. cerevisiae*

Day		Oregano, g/L				
		0	0.5	1	2	4
1	TA ^a	0.15	0.06	0.00	0.00	0.00
	C ^b	7.3×10^7	5.3×10^7	6.0×10^4	2.6×10^2	1.9×10^2
2	TA	0.97	2.27	1.85	0.09	0.08
	C	7.1×10^7	2.2×10^8	2.5×10^8	3.1×10^6	$<10^1$
3	TA	1.31	3.28	3.37	2.31	0.03
	C	4.4×10^7	2.3×10^8	3.3×10^8	1.5×10^8	1.4×10^1
4	TA	1.52	3.64	3.67	2.93	0.11
	C	4.1×10^7	1.5×10^8	4.1×10^7	4.3×10^7	3.1×10^1
5	TA	1.72	3.80	3.77	3.16	0.13
	C	2.1×10^7	2.9×10^7	1.4×10^7	7.0×10^6	$<10^1$
6	TA	1.76	3.85	3.79	3.20	0.09
	C	2.1×10^7	2.3×10^7	1.1×10^7	4.4×10^7	$<10^1$
7	TA	1.85	3.93	3.85	3.23	0.09
	C	—	—	—	—	$<10^1$

^a TA = Titratable acidity produced, ml.

^b C = Bacterial count/ml. The initial bacterial count was 1.1×10^4 cells/ml.

the essential oil of Greek and Mexican oregano, respectively. Both carvacrol and thymol have been reported to possess strong antibacterial activity (Katayama and Nagai, 1960; Kellner and Kober, 1955). In view of these facts, it appears likely that carvacrol and thymol may be responsible for the inhibition of the starter culture bacteria observed in the present study.

To remove the essential oil fraction, oregano was extracted with organic solvents, and the insoluble residues were tested for their effect on *L. plantarum* and *P. cerevisiae*. Extraction of oregano with petroleum ether, chloroform, or methanol was very effective in removing the inhibitory factor (Table 3). Bacterial growth was not inhibited while acid production was greatly enhanced in media containing residues equivalent to 8 g/L oregano, although this concentration of oregano is bactericidal to both organisms. Besides the essential oil and fixed oil fractions, much of the pigment material of oregano is also removed by solvent extraction. Alcohols are particularly effective in extracting green pigments insoluble in nonpolar solvents.

All soluble components were removed from oregano by successive washes with chloroform, ethanol, and water at room temperature. Even though the aqueous extract of oregano was stimulatory (Fig. 8), the insoluble residue, R, strongly stimulated acid production by *L. plantarum* (Fig. 10). After 7 days of incubation, the titratable acidity of the culture containing residue R, equivalent to 4 g/L oregano was 3½ times that of a control without oregano, while bacterial counts were similar. In the presence of untreated oregano, acid production began after 4 days because of inhibited bacterial growth. Apparently, the stimulatory factor of oregano is either only slightly soluble in water and is not completely removed even by extensive washing or is only slowly released on contact with water. We are presently continuing our effort to isolate and characterize the stimulatory factor.

We have found only a few reports in the literature indicating that spices possess components that may stimulate metabolic activities of microorganisms. Corran and Edgar (1933), reporting on the effect of various spices on yeast fermentation, suggested that the herbs thyme, bay leaf, marjoram, savory, and rosemary contain a yeast stimulant, since the loss of glucose in the media containing these herbs was significantly larger than in the control medium. Wright et al. (1954) showed that several spices

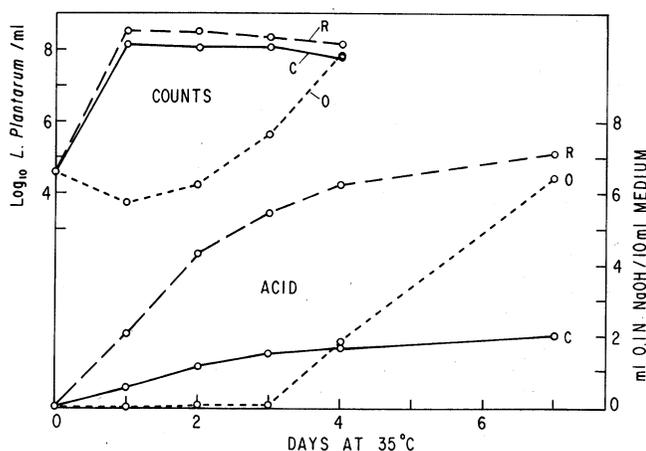


Fig. 10—Effect of oregano before and after extraction with chloroform, ethanol, and water on growth of and acid production by *L. plantarum*. C = control, O = oregano, 4 g/L; R = oregano, 4 g/L, after extraction with chloroform, ethanol, and water.

at low concentrations induced a considerable stimulation of gas production during yeast fermentation but that the enhanced gas production in the presence of spices was not due to accelerated yeast cell proliferation. They also showed that the spices retained their stimulatory properties after extraction with ether, autoclaving, or prolonged boiling. These findings are in agreement with our observations with oregano as well as other spices.

In summary, our results indicate that bacteria may be inhibited by oregano added to foods but may also develop resistance to the inhibitory effect of the spice.

REFERENCES

- Beuchat, L.R. 1976. Sensitivity of *Vibrio parahaemolyticus* to spices and organic acid. *J. Food Sci.* 41: 899.
- Blum, H.B. and Fabian, F.W. 1943. Spice oils and their components for controlling microbial surface growth. *Fruit Product J.* 22: 326.
- Calzolari, C., Stancher, B., and Marletta, G.P. 1968. Origanum oils and their investigation by gas-chromatographic and infrared spectroscopic analysis. *Analyst* 93: 311.
- Chopra, C.L., Bhatia, M.C., and Chopra, I.C. 1960. In vitro antibacterial activity of oils from Indian medicinal plants. *J. Am. Pharm. Assoc.* 49: 780.
- Collier, W.A. and Nitta, Y. 1930. Über die Wirkung ätherischer Öle auf verschiedene Bakterienarten. *Z. Hyg. Infektionskrankh.* 111: 301.
- Corran, J.W. and Edgar, S.H. 1933. Preservative action of spices and related compounds against yeast fermentation. *J. Soc. Chem. Ind.* 52: 149T.
- Dold, H. and Knapp, A. 1948. Antimikrobielle Wirksamkeit ätherischer Öle. *Z. Hyg. Infektionskrankh.* 128: 696.
- Fabian, F.W., Krehl, C.F., and Little, N.W. 1939. The role of spices in pickled food spoilage. *Food Res;* 4: 269.
- Ikeda, R.M., Stanley, W.L., Vannier, S.H., and Spitler, E.M. 1962. The monoterpene hydrocarbon composition of some essential oils. *J. Food Sci.* 27: 455.
- Julseth, R.M. and Deibel, R.H. 1974. Microbial profile of selected spices and herbs at import. *J. Milk Food Technol.* 37: 414.
- Katayama, T. and Nagai, I. 1960. Chemical significance of the volatile components of spices from the food preservative viewpoint. 4. Structure and antibacterial activity of some terpenes. *Nippon Suisan Gakkaishi* 26: 29. [Chem. Abstr. 55: 6720a (1961)].
- Kellner, W. and Kober, W. 1955. Possibilities of the use of ethereal oils for room disinfection. 2. *Arzneimittel-Forsch.* 5:224. [Chem. Abstr. 49: 11091f (1955)].
- Kissinger, J.C. and Zaika, L.L. 1978. Effect of major spices in Lebanon bologna on acid production by starter culture organisms. *J. Food Prot.* 41: 429.
- Koedam, A. 1977. Antimikrobielle Wirksamkeit ätherischer Öle. Eine Literaturarbeit 1960—1976. *Riechst. Aromen Körperpflege.* 27(1): 6; 27(2): 36.
- Maruzzella, J.C. and Balter, J. 1959. The action of essential oils on phytopathogenic fungi. *Plant Disease Reprtr.* 43: 1143.
- Maruzzella, J.C. and Freundlich, M. 1959. Antimicrobial substances from seeds. *J. Am. Pharm. Assoc.* 48: 356.
- Maruzzella, J.C. and Henry, P.A. 1958. The in vitro antibacterial activity of essential oils and oil combinations. *J. Am. Pharm. Assoc.* 47: 294.
- Maruzzella, J.C. and Liguori, L. 1958. The in vitro antifungal activity of essential oils. *J. Am. Pharm. Assoc.* 47: 250.
- Maruzzella, J.C., Reine, S., Solat, H., and Zeitlin, A. 1963. The action of essential oils on phytopathogenic bacteria. *Plant Disease Reprtr.* 47: 23.
- Maruzzella, J.C. and Sicurella, N.A. 1960. Antibacterial activity of essential oil vapors. *J. Am. Pharm. Assoc.* 49: 692.
- Rhyu, H.Y. 1979. Gas chromatographic characterization of oregano and other selected spices of the Labiate family. *J. Food Sci.* 44: 1373.
- Stahl, W.H., Skarzynski, J.N., and Voelker, W.A. 1969. Differentiation of geographic origin of spices. 2. Oregano by gas chromatography and thin-layer chromatography. *J. Assoc. Off. Anal. Chem.* 52: 1184.
- Webb, A.H. and Tanner, F.W. 1945. Effect of spices and flavoring materials on growth of yeasts. *Food Res.* 10(4): 273.
- Wright, W.J., Bice, C.W., and Fogelberg, J.M. 1954. The effect of spices on yeast fermentation. *Cereal Chem.* 31: 100.
- Zaika, L.L. and Kissinger, J.C. 1979a. Effects of some spices on acid production by starter cultures. *J. Food Prot.* 42: 572.
- Zaika, L.L. and Kissinger, J.C. 1979b. Inhibitory and stimulatory effects of spices and herbs on lactic acid starter cultures. 39th Annual Meeting, Institute of Food Technologists, St. Louis, MO, June 10—13. Paper #367.
- Zaika, L.L., Zell, T.E., Palumbo, S.A., and Smith, J.L. 1978. The effect of spices and salt on fermentation of Lebanon bologna-type sausage. *J. Food Sci.* 43: 186—189.

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titratable acidity was doubled after 7 days of fermentation. In the presence of 2 g/L oregano, *P. cerevisiae* at first declined in numbers from 1.1×10^4 to 2.6×10^2 cells/ml but later grew and produced acid.

The results with sublethal concentrations of oregano suggested that the starter culture bacteria may become resistant to the toxic effects of oregano. A method was developed to test for acquired resistance (Fig. 1). Starter cultures were inoculated into a medium containing a sublethal quantity of oregano—3 g/L was used for both organisms. When acid production began after a delay of several days, bacteria from the oregano-containing sample was inoculated into a medium containing 3 g/L oregano, O₁, and a control medium, C₁. Bacteria growing in these samples were then subcultured into new sets of the same media (Fig. 1).

The results from the initial inoculation with *L. plantarum* are shown in Figure 2. Bacterial counts increased from 2.2×10^4 to 1.2×10^7 cells/ml after 1 day in the control medium, C. In the oregano-containing medium, O, the count at first declined then gradually increased to 3.1×10^7 cells/ml after 3 days, at which time acid production also began. After 3 days, bacteria that had been exposed to

oregano were inoculated into a new set of media, a control, C₁, and a medium containing 3 g/L oregano, O₁ (Fig. 3). Bacterial growth was no longer inhibited by the presence of oregano. Titratable acidity in O₁ increased even after 1 day of incubation and was 5.3 ml compared to 0.8 ml for the control after 5 days. After 1 day of incubation, both C₁ and O₁ were subcultured into fresh control and oregano-containing media. Bacterial counts and titratable acidities were measured over a period of 6 days. Both sets of curves, C₂, O₂ and C₃, O₃, respectively, (Fig. 4) were similar, showing no inhibition of growth of *L. plantarum* but strong stimulation of acid production in the oregano-containing media.

P. cerevisiae was subcultured in a similar manner. Figure 5 shows the effect of the initial exposure of *P. cerevisiae* to a sublethal concentration of 3 g/L oregano. The bacterial population in the oregano-containing medium, O, at first decreased from 3.1×10^4 to 1.8×10^2 cells/ml after 3 days, but then increased to 4.9×10^7 cells/ml after 5 days

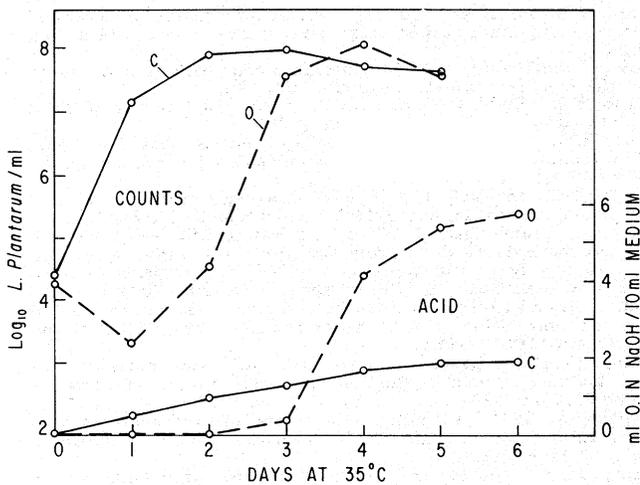


Fig. 2—Growth of and acid production by *L. plantarum* in a control medium, C, and in a medium containing 3 g/L oregano, O.

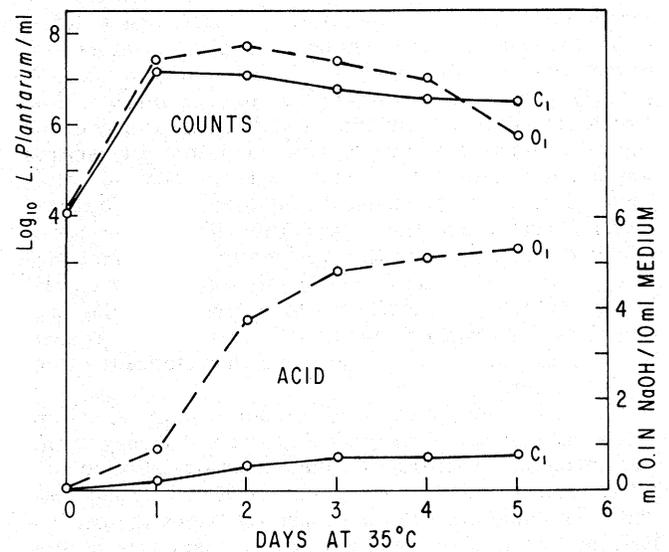


Fig. 3—Growth of and acid production by *L. plantarum* subcultured after 3 days from O. C₁ = control medium; O₁ = medium containing 3 g/L oregano.

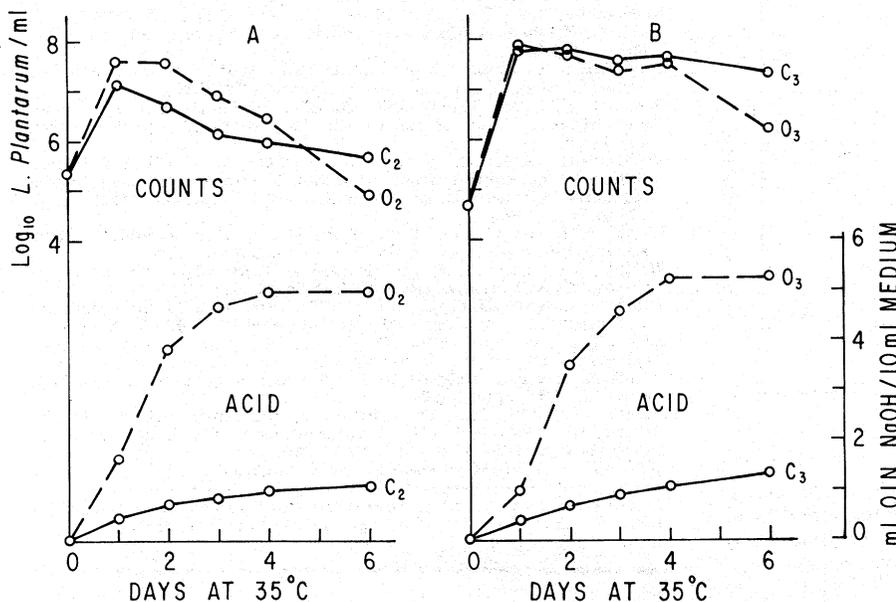


Fig. 4—Growth of and acid production by *L. plantarum* subcultured after 1 day from O₁ (Fig. 4a) and from C₁ (Fig. 4b). C₂, C₃ = control medium; O₂, O₃ = medium containing 3 g/L oregano.

with initiation of acid production. At this point, bacteria from the oregano-containing medium, O, were inoculated into a new set of media with and without oregano (Fig. 6). As in the case of *L. plantarum*, *P. cerevisiae* was no longer inhibited by 3 g/L oregano. Bacterial counts of the control,

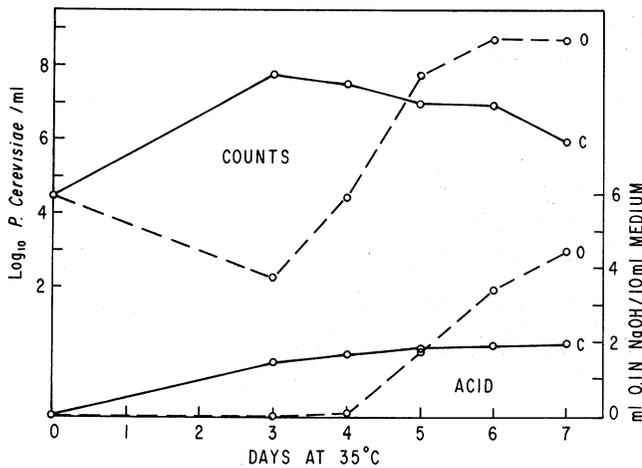


Fig. 5—Growth of and acid production of *P. cerevisiae* in a control medium, C, and in a medium containing 3 g/L oregano, O.

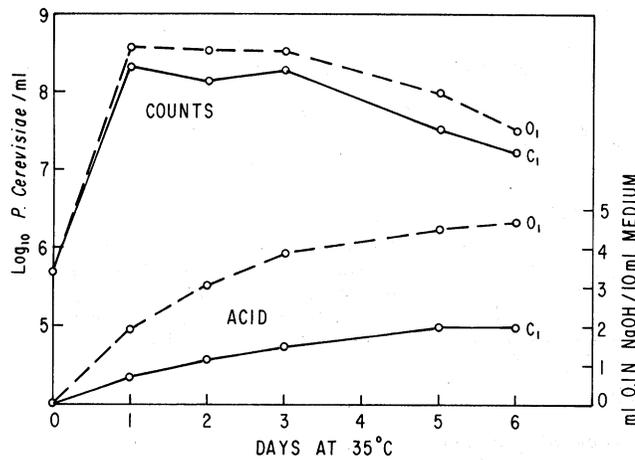


Fig. 6—Growth of and acid production by *P. cerevisiae* subcultured after 5 days from O. C₁ = control medium; O₁ = medium containing 3 g/L oregano.

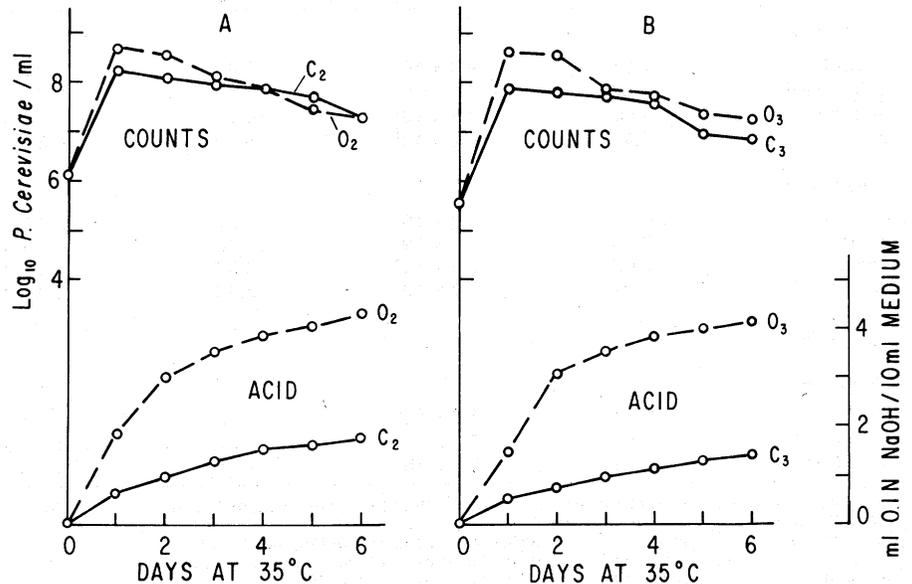


Fig. 7—Growth of and acid production by *P. cerevisiae* subcultured after 1 day from O₁ (Fig. 7a) and from C₁ (Fig. 7b). C₂, C₃ = control medium; O₂, O₃ = medium containing 3 g/L oregano.

C₁, and of the oregano-containing sample, O₁, reached 10⁸ cells/ml after 1 day and were similar throughout the incubation period. Titratable acidity of O₁ was higher than that of C₁ even after only 1 day of fermentation. After 1 day of incubation, both C₁ and O₁ were subcultured into a new set of media with and without oregano. Both sets of curves, C₂, O₂ and C₃, O₃, respectively, showing bacterial counts and titratable acidities for samples inoculated from C₁ and O₁ (Fig. 7) were similar. In the presence of oregano, bacterial growth was not inhibited and acid production was enhanced. These experiments indicate that the starter culture bacteria had become resistant to the toxic action of oregano during their initial exposure to the spice, and that this resistance was retained while the bacteria were grown in a control medium, C₁. An additional step in the subculturing scheme was carried out with similar results.

Oregano apparently contains two factors, one that inhibits the growth of lactic acid bacteria and one that stimulates acid production by these microorganisms. To gain information about the nature of the inhibitory and the stimulatory factors of oregano, the following experiments were carried out. Comparison of the response of *L. plantarum* to 4 g/L oregano and to the water-soluble components equivalent to this amount of the spice showed that both the inhibitory and the stimulatory factors of oregano are at least partly soluble in water (Fig. 8). Acid production was delayed at least 4 days in the presence of 4 g/L oregano, but only 1 day in the presence of the aqueous extract. The aqueous extract of oregano also stimulated acid production by *L. plantarum*. In the early stages of incubation bacterial counts decreased in the presence of oregano, while they increased in the presence of the aqueous extract, but at a slower rate than in the control medium.

In the presence of 4 g/L oregano that had been autoclaved in suspension with the liquid medium, the growth of *L. plantarum* was not inhibited and acid production was greatly enhanced (Fig. 9). By comparison, untreated oregano inhibited acid production for at least 4 days. Thus, autoclaving removed or destroyed the inhibitory components while the factor responsible for stimulation of acid production was stable to heat.

The inhibitory component of oregano most likely resides in the essential oil fraction. We have found no reports in the literature concerning the effect of oregano or its essential oil on starter culture bacteria. The essential oil of oregano has been tested for antimicrobial activity against a variety of microorganisms, mostly pathogenic.

Out of a large number of essential oils tested by Maruzzella and his co-workers oregano oil was among the most antibacterial. Good antimicrobial activity was obtained against fungi (Maruzzella and Liguori, 1958; Maruzzella and Balter, 1959) and bacteria (Maruzzella and Henry, 1958; Maruzzella et al., 1963). Essential oil vapors were also found to be inhibitory to bacteria, and oregano oil was among the six most active of the 133 oils tested (Maruzzella and Sicurella, 1960). Oregano oil inhibited *Vibrio parahaemolyticus* (Beuchat, 1976) and yeasts (Blum and Fabian, 1943). Chopra et al. (1960) reported that oregano oil had some activity against bacteria. The composition of oregano essential oil has been studied (Rhyu, 1979; Calzolari et al., 1968; Ikeda et al., 1962). Although carvacrol is the major constituent, considerable amounts of thymol may also be present. Stahl et al. (1969) determined that the ratio of the concentrations of carvacrol to thymol in Greek oregano is approximately 7:1, whereas in Mexican oregano it is 1:1. Data presented by Rhyu (1979) indicate that carvacrol and thymol constituted 40–85% and 30–57% of

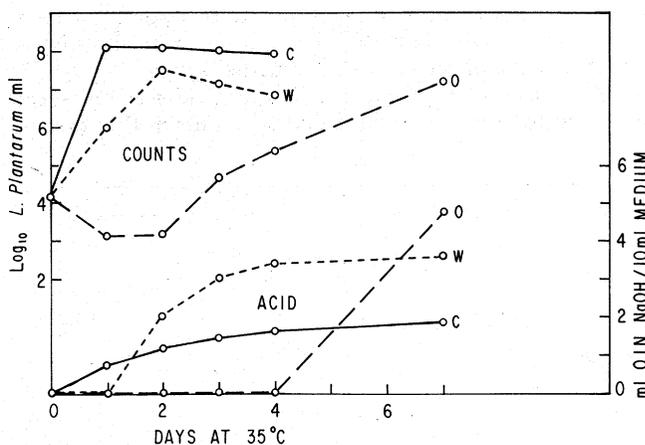


Fig. 8—Effect of oregano and its aqueous extract on growth of and acid production by *L. plantarum*. C = control; O = oregano, 4 g/L; W = aqueous extract equivalent to 4 g/L oregano.

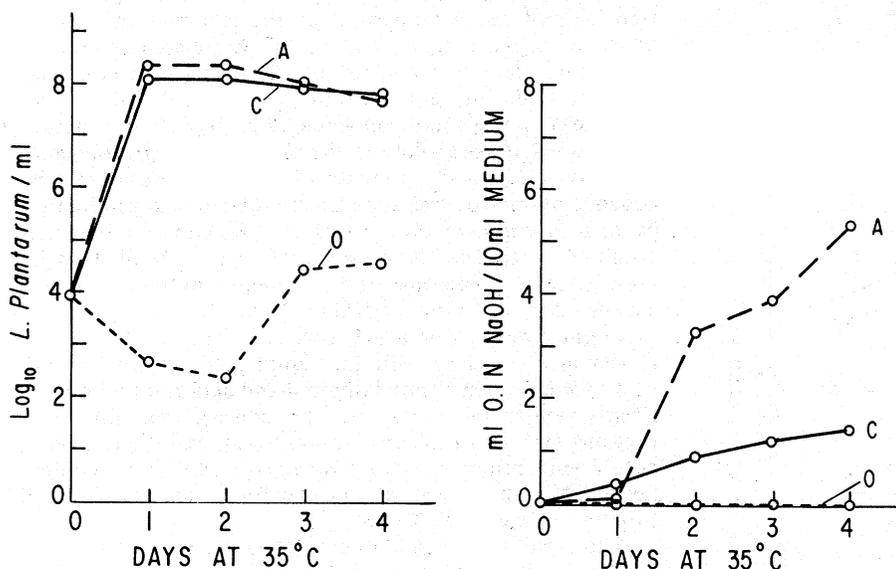


Fig. 9—Effect of autoclaving of oregano on growth of and acid production by *L. plantarum*. C = control; O = oregano, 4 g/L; A = oregano, 4 g/L, autoclaved in suspension with the medium.

Table 3—Effect of solvent extracted oregano^a on growth of and acid production by starter cultures.

	Incubation time at 35°C					
	Day 1		Day 2		Day 4	
	TA ^b	count/ml	TA	count/ml	TA	count/ml
<i>L. plantarum</i> ^c						
control	1.15	2.6 × 10 ⁷	1.45	8.0 × 10 ⁶	1.65	2.9 × 10 ⁶
oregano-PE	4.37	6.1 × 10 ⁸	6.47	8.3 × 10 ⁸	9.13	2.0 × 10 ⁸
oregano-C	4.66	7.1 × 10 ⁸	6.71	9.0 × 10 ⁸	9.45	1.5 × 10 ⁸
oregano-M	4.45	8.4 × 10 ⁸	6.49	8.3 × 10 ⁸	8.99	1.5 × 10 ⁸
<i>P. cerevisiae</i> ^d						
control	0.27	3.6 × 10 ⁷	0.91	5.1 × 10 ⁷	1.43	4.6 × 10 ⁷
oregano-PE	0.89	3.6 × 10 ⁸	2.98	4.1 × 10 ⁸	4.59	2.6 × 10 ⁸
oregano-C	2.05	5.2 × 10 ⁸	3.37	3.3 × 10 ⁸	4.51	1.3 × 10 ⁸
oregano-M	2.07	8.7 × 10 ⁸	3.32	4.8 × 10 ⁸	3.92	1.7 × 10 ⁸
<i>L. plantarum</i> ^e + <i>P. cerevisiae</i>						
control	0.73	1.0 × 10 ⁸	1.15	9.1 × 10 ⁷	1.69	6.6 × 10 ⁷
oregano-PE	2.58	1.1 × 10 ⁸	5.37	8.9 × 10 ⁷	6.47	7.0 × 10 ⁶
oregano-C	3.68	2.7 × 10 ⁸	7.38	2.9 × 10 ⁸	10.35	6.6 × 10 ⁷

^a Oregano, 8 g/L, after extraction with petroleum ether (PE), chloroform (C), or methanol (M).

^b TA = Titratable acidity produced, ml.

^c All samples initially contained 2.9 × 10⁴ cells/ml of Lactacel DS starter culture.

^d All samples initially contained 7.0 × 10⁴ cells/ml of Lactacel starter culture.

^e All samples initially contained 8.0 × 10⁴ cells/ml of Lactacel MC starter culture.