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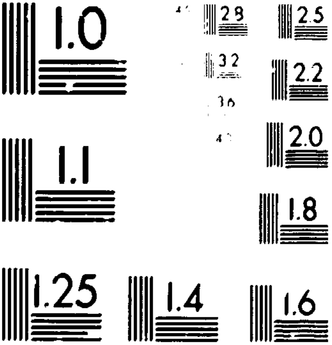

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ECOLOGY OF LACTIC FERMENTATIONS OF STARCHY FOODS*

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CONTENTS

I. BACKGROUND

Lactic starch fermentations are important for the conservation and transformation of staple food, such as, cereal grains and edible roots, in many tropical countries. Therefore, it is important to understand the ecology of this process for future industrial developments e.g., for infant food and traditional food industries.

In this study, we have chosen traditional fermentations such as, sauerkraut, "pozol" (fermented maize dough) and fresh cow dung as microbial sources. Using conventional microbial techniques 76 lactic bacterial strains were isolated and classified according to genera (Lactobacillus, Streptococcus, Leuconostoc or Pediococcus) and type of metabolic reaction: homo or heterolactic. Their ability to use starch, glucose, ammonium sulphate and peptone, as sole carbon or nitrogen sources, was analyzed, using both mesophilic or thermophilic culture conditions.

Statistical comparisons among those strains indicated the importance of the nature of primary microbial source (sample for inoculum) as a leading factor of such ecology. For example, thermophilic streptococci were predominant in cow dung but more even distributions among genera were found in the other sources.

It is possible to obtain thermophilic amilolytic homolactic bacteria with appropiate choice of samples and culture media. This type of result could be used for developing new industrial processes based on traditional or agricultural fermentations. The optimization of lactic fermentation of enriched cassava meal (by Aspergillus niger) was used as an example of alternative procedures for inexpensive conservation of feedstuffs.

Lactic-acid-fermentation. Starch. Food fermentation. Silage. Traditional fermentation.

II. INTRODUCTION

Application of biotechnology to food fermentations could help to solve some pressing problems of developing nations, such as, reducing food waste, enriching staple foods and changing the flavour of raw materials. There are many traditional fermentations of non-dairy substrates, such as cereal grains, vegetables or starchy roots. This kind of fermentation could be the basis for isolating new strains of lactic bacteria to be used later in the manufacturing of new types of fermented foods or feeds.

A typical example of a traditional fermentation of Mexico with promising features for industrialization is the so called "pozol". It is made by spontaneous lactic acid fermentation of lime cooked maize dough called "nixtamal". Pozol was found to have a protein with superior nutritional value with respect to unfermented maize and this was shown to be due to increasing amounts of tryptophane and lysine (Cravioto et al., 1955). Herrera and Ulloa (1970) have characterized the mycoflora of pozol but there is no published information on the type of bacteria present in this material, although, these authors have indicated the presence of a large amount of lactic bacteria. Fields et al.(1981) have confirmed the presence of lactic bacteria in the spontaneous fermentations of maize dough. Among the interesting properties of pozol fermentation is the presence of some kind of nitrogen fixing microorganisms which can also help to increase the net amount of protein (Ulloa et al., 1977). Cravioto et al. (1955) concluded that pozol was an example of the empirical wisdom developed by indigenous cultures of Mexico in relation to food processing and conservation.

Present market needs of tropical countries include the supply of increasing amounts of concentrated feeds for poultry and swine production. This has been met through competing use of cereal grains in relation to direct human consumption of staple food or through importation of feed grains. An alternative solution would be the use of tropical products such as cassava roots but they have to be enriched with additional protein in order to have an appropiate proximal composition. This can be done by growing moulds in semisolid raw materials (Raimbault, 1981; Hesseltine, 1983) and the resulting humid product can be stabilized by lactic acid fermentation. This case can be used as an illustration of a new process of lactic fermentation that can be developed using lactic bacteria isolated from traditional food fermentations, such as pozol. In order to do so it seems appropiate to study the comparative ecology of lactic acid fermentation on starch, using different microbial sources, for example: pozol, sauerkraut or cow dung.

Comparative biochemical and physiological studies of lactic bacteria could be useful in order to select new industrial strains for food or feed fermentation of starch. Among the important features to be analyzed are: ability to use starch as the main carbon source, tolerance to heating, and the ability to use simple nitrogen sources such as mineral salts instead of complex organic sources such as polypeptides. Ideally a good industrial strain should be able to use starch, grow on mineral salts as nitrogen source and resist moderate or high levels of heating (above 40 C).

In this work a comparative study has been done on the microbiology of three different microbial sources with emphasis on the screening of lactic bacteria with the aforementioned phyisiological traits.

III. BACKGROUND ON TRADITIONAL FERMENTATIONS OF MAIZE IN MEXICO

Traditional fermentations of Latin America have received less attention as compared to food fermentations of Asia. (Wang and Hesseltine, 1981; Van Veen and Steinkrauss, 1970; Shallenberger et al., 1967; both cited by Pederson, 1979; Hesseltine, 1983). In Mexico the group of Herrera and Ulloa (Instituto de Biologia, UNAM) have made a number of studies of the traditional fermentations maize (Cruz-Ulloa and Ulloa, 1973). Special attention was paid to "pozol" and "tesguino". Pozol, named after the indigeneous word "pozolli" or foamy is presently consumed in the South Easter region of Mexico specially by ethnic groups of Mayan ancestry. It is a fermented sour dough of maize. According to Ulloa (1974) the dough is prepared by boiling the kernels with lime and partially grinding them until a granular paste is obtained. Small balls are enveloped with banana leaves and left to have spontaneous fermentation at room temperature (25 to 35 C). The fermented product is consumed diluted with water and condimented with different spices.

Cravioto et al. (1955) found a remarkable improvement of the nutritional value of maize during pozol fermentation. Net protein increase was observed which could not be explained by apparent enrichment due to starch consumption. Ulloa et al. (1977) showed the presence of nitrogen fixing reactions using acetylene reduction techniques and isolated a yet unclassified bacterium with nitrogenase capacity.

Munoz and Viniegra-Gonzalez (1981) observed that lactic acid could be a good substrate for free living bacteria with the ability of fixing atmospheric nitrogen (Azotobacter chrococcus) and suggested the possibility that lactic fermentation of pozol was coupled to nitrogen fixation. Aminoacid composition of pozol protein was found to be richer in lysine and trytophane as compared to maize protein (Cravioto et al., 1955), indicating that lactic fermentation could be an interesting way of changing the nutritional value of staple food.

Microbial studies of pozol (Ulloa and Herrera, 1981) found a large variety of moulds, yeasts and lactic bacteria. Moulds and yeasts were found on the surface of pozol balls. Lactic bacteria were found inside . This result has suggested that this material could be an interesting microbial source for isolating new industrial strains to be later used in food transformations.

Tesguino (form Nahuatl language, "tecuin", or heart thumping) is an alcoholic beverage made by liquid fermentation of maize wort, fortified with raw sugar (Ulloa and Herrera, 1981; Cruz-Ulloa and Ulloa, 1973). It is a raw product composed of the fermented wort and grain residues together with microbial biomass. Different yeasts have been found in tesguino, such as, Candida and Pichia spp. in addition to Bacillus megaterium (Ulloa et al. 1974). This is a traditional example of the know-how of Mexican cultures for developing different types of maize fermentations (lactic in pozol or alcoholic in tesguino) using different empirical formulation. A recent review on this subject (Viniegra-Gonzalez and Gomez, 1983) suggested the idea that changes in C/N ratios can induce different metabolic patterns of traditional fermentations. Alcoholic fermentation would be produced with C/N higher than 30 and lactic fermentation otherwise. This has been substantiated by experimental work on the ecology of mixed heterogeneous cultures and was discussed by Saucedo et al. (1984).

IV. TAXONOMIC DISTRIBUTION OF LACTIC BACTERIA FROM TRADITIONAL AND WASTE MATERIALS

The original purpose of this work was to isolate new strains of lactic bacteria with the features described in the introduction. The hypothesis was that physiological behaviour of a given microbial population would depend on the nature and environmental conditions of the starting material more than on the taxonomic distribution of lactic bacteria (Viniegra-Gonzalez and Gomez, 1984). In order to test such hypothesis, three different types of samples were chosen: pozol, sauerkraut and cow dung. All three are known to be a good source of lactic microorganisms but with entirely different physiological and nutritional properties (Viniegra-Gonzalez and Gomez, 1984).

The use of conventional microbiological techniques lead to the isolation of 72 colonies of lactic bacteria from the following starting materials: sauerkraut (prepared at 37 or 42 C), traditional pozol obtained from Tabasco City (Mexico) and fresh cow dung. Tesguino was discarded as starting material because most of the isolated lactic strains were rather fastidious for culture reproduction.

The taxonomic distribution of genera is indicated in Table I. On the average, 51% of all strains belong to Lactobacillus , 24% to Leuconostoc , 13% to Streptococcus and 12% to Pediococcus . In relation to the fermentation pattern, evaluated by means of gas chromatography of the fermentation products, 54% were hetero fermenters and 46% homo fermenters.

Taxonomic distribution in each given sample had considerable differences. Cow dung had a large majority of Streptococcus (75%) and none of Leuconostoc and Pediococcus . Pozol had: Lactobacillus , 56%; Leuconostoc . 39%. and Streptococcus . 6%. Taxonomic microbial distribution in sauerkraut depended on the temperature at which the process was carried out. At 37 C, Leuconostoc were more abundant (37%) than at 42 C (19%). Pediococcus from sauerkraut depended also on process temperature since they dissapeared at 42 C. These results seem to indicate the important influence of the starting material on the taxonomic distribution of lactic bacteria (Table I). In particular. pozol seems to be a good source for the isolation of Lactobacillus which may use starch as a sole source of carbon. because of the large proportion of this substrate in the maize kernels.

A more detailed study on the physiological features of each kind of strain. indicated that among the Lactobacillus isolated from pozol. a large fraction grew on starch as a sole carbon source and nearly a fifth of all Lactobacillus could grow on starch using ammonium sulphate as a nitrogen source supplemented with small amounts of yeast extract (less than 10% of all nitrogen). Most of those Lactobacillus strains were heterlolactic and practically none of the homolactic could grow in ammonium sulphate and starch.

Thermotolerance. evaluated by the proportion of colonies able to grow at 42 C. as compared to a basal temperature of 37. was found to be a more common feature of cow dung homolactic streptococci with respect to the heterolactic bacteria of pozol and sauerkraut (Figs. 1 and 2).

These results suggest that cow dung might be a valuable source of bacteria for silage. that is for the fermentation of animal feeds but it is clear that sanitary and esthetic considerations might reduce their potential for food fermentations. Fortunately enough. some of the very few homolactic and thermotolerant Lactobacillus from pozol were found to have a good acidogenic capacity using ammonium sulphate and starch as the basal culture medium. Those rare strains have been used in order to develop new fermentation processes for food and feed fermentation.

V. OPTIMIZATION OF STARCH LACTIC FERMENTATION

A number of experiments have been done in our laboratory in order to optimize lactic fermentation of starch. We were particularly interested on the possibility of using lactic fermentation in order to preserve enriched cassava by the biomass of Aspergillus niger previously grown on cassava meal. The objective was to develop a new two-step fermentation process for cassava meal transformation into a well balanced feedstuff having more than 15% protein and a high level of digestible carbohydrates to be consumed by monogastric animals (swine and poultry). Lactic fermentation was thought to be a cheaper stabilization process as compared to heat oven drying of enriched cassava but it was necessary to show the technical feasibility of lactic fermentation of samples having no more than 65% of humidity and with little requirements on process control.

Optimization studies were done using a modified Box-Wilson matrix array of seven variables indicated in Table 2. Those were. humidity. inoculation with starter culture. addition of calcium hydroxide. modification of temperature. addition of molasses. urea and cellulose fibers. The specific experimental design is shown in Table 3. It is a truncated factorial design which explores some of the interactions with the assumption that non-linear terms are negligible as a first approximation. This kind of experimental design is commonly used for the optimization of fermentation processes.

The results obtained indicated that the addition of calcium hydroxide. starter culture and cellulose were the major factors involved in the optimization of this type of silage fermentation (Table 4) using the values of each factor indicated in Tables 2 and 3.

The response R to those factors could be explained by the following linear regresion:

$$R = Fo + b1*A + b2*B + ... + bi*I$$

R= observed value

Fo= residual effect of non-studied factors

b1.....bi = linear regression coefficients for each studied factor.

A.....I = sense of each variation (+1 or -1) for each studied factor around a given intermediate value (arbitrary origin or "zero").

The experimental results for lactic acid production (g/l) were:

$$R = 1.48 + 0.41(\%H_2O) + 0.56(inoculum) + 1.19(Ca(OH)_2) + 0.12(temperat.) - 0.10(molasses) - 0.26(urea) + 0.58(cellulose).$$

Those results seem to suggest that the following possitive factors are to be considered for optimization.

1) Lactic acid neutralization was a major control factor of lactic fermentation (80% of variation). This is known since long time ago and has been explained in terms of the ionization of lactic acid into lactate. being lactic acid the responsable chemical species for product inhibition of tnis process (Luedeking and Piret. 1959)

2) Cellulose might have changed the texture of the fermentation mixture (39.2% of variation). either by adsorbing bacteria. products or substrates.

3) Starter (inoculum) addition of non-sterile raw materials seems to be not an essential but a benefitial factor of lactic fermentation (38% of variation). This is due to the ubiquitous nature of lactic bacteria in edible samples.

4) Increasing levels of humidity favored lactic fermentation (27% of variation)

5) Increasing temperature. above 28.5 C. increased lactic fermentation slightly (8% of variation).

The negative influences for optimization were:

6) Excess alkalinity. perharps produced by the addition of urea. transformed into $(NH_4)OH$. might have been harmful to lactic fermentation (-17.6% of variation)

7) Excess sugar added with molasses, might have been a distorting factor that changed the C/N ratio of readily fermentable substrates (-6% of variation).

This exercise is given here in order to illustrate the kind of experimental work which could be necessary for process development of new fermented products using traditional fermentations as starting microbial population and new valuable substrates as raw materials. It is worth mentioning that validity of multiple regression coefficients is considered only in the limited range of observation. Perharps the value and sign of those coefficients would change outside such a small range. For example, a number of results indicate the positive effect of urea addition (Viniegra-Gonzalez and Gomez, 1984). In this particular example it is only possible to ascertain that urea added together with calcium hydroxide seems to be a negative factor. Furthermore. the addition of readily fermentable sugars could be benefitial in other experimental conditions. In this case, it was found a negative factor.

Percentage calculations on the various weights for each variation coefficient help to decide priorities of future experimental work. For example. it seems worthwhile to study the effect of calcium hydroxide. cellulose addition and the amount and type of starter cultures, because they seemed to have the biggest weights on the variation of lactic fermentation (80. 39 and 38%. respectively). But. temperature changes around 27-30 C did not seem to be significant: small amount of addition of molasses. neither (+8% and -6%. respectively). Intermediate factors such as humidity (+27%) and urea (-18%) could be investigated if there are enough time and resources.

Therefore. this method seems to be useful in order to assign research priorities to different alternatives of optimization. But it might not be the best way to ascertain cause-effect relationships since the experimental results are given only in terms of a phenomenological multilinear regression. For a more profound understanding of such type of relationships model testing would be required which was beyond the scope of this work.

VI. DISCUSSION

The evidence presented here seems to indicate that there is a potential use of traditional lactic fermentations as new microbial sources for starter cultures in conventional or in new process of food and feed transformation. Previous work done by the group of Herrera and Ulloa in our National University (UNAM) and older work of Cravioto et al. (1955) has provided grounds for selecting "pozol" fermentation as a very intriguing and atractive subject of research. Among the its attractive features, a) The amino acid enrichment of cereal protein by the action of lactic bacteria (Cravioto et al., 1955), b) The ability to fix atmospheric nitrogen because of the coexistence of lactic fermentation with indigenous nitrogen fixing bacteria, c) the presence of homo and heterolactic bacteria that are able to transform starch in lactic acid and their apparent minimal nutritional requirements, as indicated by their use of ammonium sulphate as the main nitrogen source, d) the possibility of isolating thermotolerant lactic bacteria.

Comparison of the lactic bacteria from pozol and those from sauerkraut and cow dung offers evidence on the importance of appropiate choice of microbial sources for the isolation of new starter cultures with specific physiological features. For example, cow dung seems to be a good source for thermotolerant homolactic streptococcii. It has the problem of coexistence with coliform bacteria which could have evolved with some degree of contamination by pathogenic phages or viruses. Therefore its use should be restricted to animal feed or chemical production, for example, silage of agricultural residues, methanogenesis from industrial food waste or continuous production of chemical feedstocks.

On the other hand the use of lactic bacteria isolated from diverse traditional fermentations could add variety for development of new food products. For example, sauerkraut seemed to be a good source for heterolactic bacteria but with less ammount of strains able to grow in starch as a sole carbon source. Pozol, in turn, being a traditional starch fermentation, provided some interesting strains for such a kind of food substrate.

Isolation of strains is only one step in the direction of new product development. It is necessary to optimize the culture medium in order to use new starters. There are factors such as level of humidity and temperature which cannot be changed very much since may depend on the process specification, for example, in silage of prefermented materials. But there are additional factors to be considered

such as sugar and titrant addition. In all those cases, the number of potential interactions is very large and it seems helpful to have optimal experimental designs with a minimal number of observations. The truncated factorial design presented in this work seemed to be consistent with the accumulated work on the ecology of lactic fermentation, providing sometimes, new insights on the importance of additional factors, such as, the apparent benefitial effect of fiber addition.

In summary, evidence is presented on the potential of traditional fermentations for isolating new industrial cultures for food and feed production and a method is suggested on how to proceed for optimal experimental design for process development. This type of work could lead to up-graded traditional proceses with new markets in developing nations.

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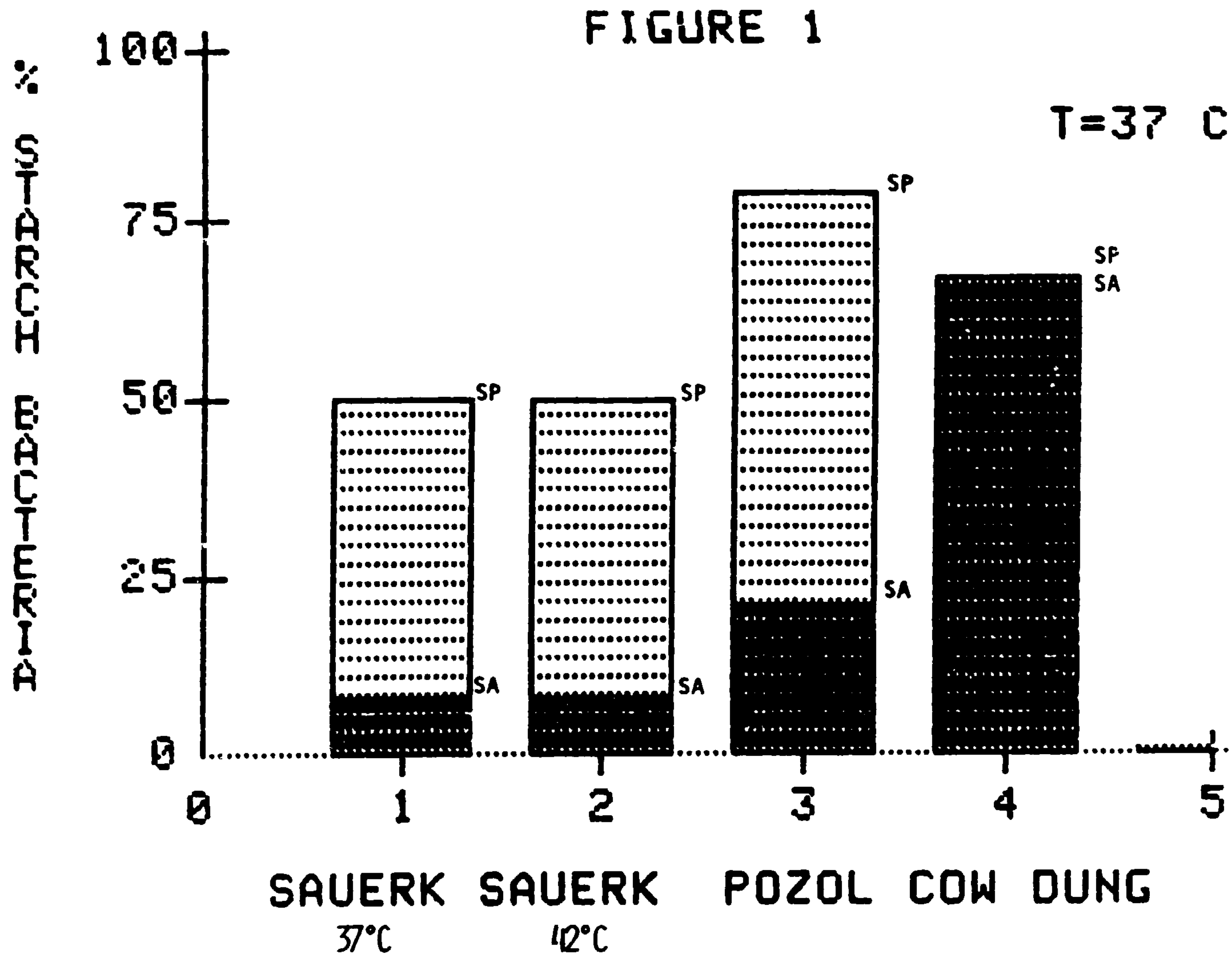
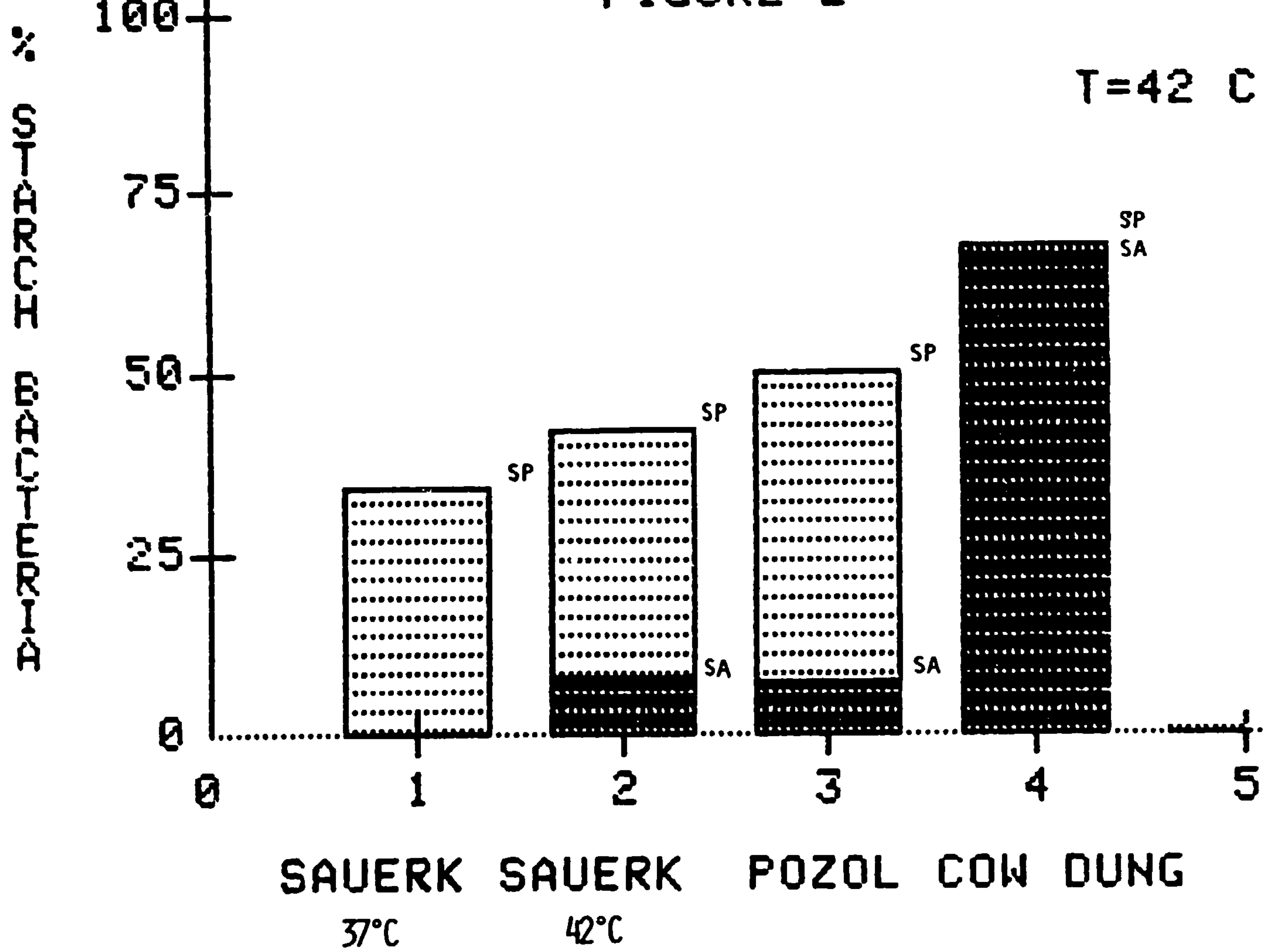


SP = STARCH WITH PEPTONE

SA = STARCH WITH AMMONIA

FIGURE 2



SP = STARCH WITH PEPTONE

SA = STARCH WITH AMMONIA

FIGURE 3

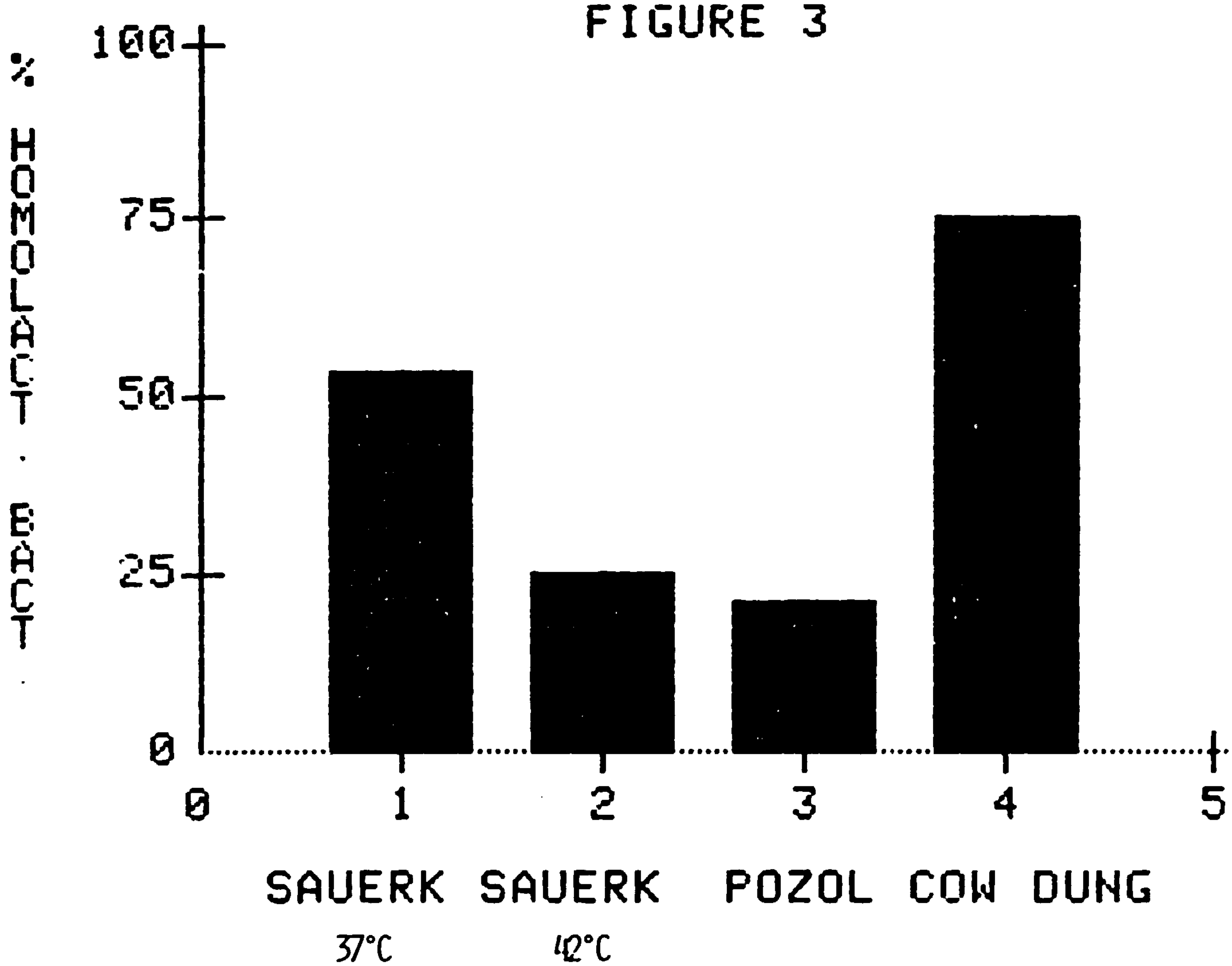

% HOMOLACT. BACT.
100
75
50
25
0
0
1
2
3
4
5
SAUERK
37°C
SAUERK
42°C
POZOL
COW DUNG

TABLE 1

LACTIC BACTERIA DISTRIBUTION.

SOURCE	STRAIN No.	LEUCONOSTOC	LACTOBACILLUS	STREPTOCOCCUS	PEDIOCOCCUS	HETERO	HOMO
SAUERKRAUT 37°C	38	14 %	24 %	0 %	12 %	24 %	26 %
SAUERKRAUT 42°C	12	3 %	13 %	0 %	0 %	12 %	4 %
POZOL	14	7 %	10 %	1 %	0 %	14 %	4 %
COW DUNG	12	0 %	4 %	12 %	0 %	4 %	12 %
	72	24 %	51 %	13 %	12 %	54 %	46 %

TABLE 2

UPPER AND LOWER LEVELS FOR FACTORS STUDIED		
FACTORS	LEVEL +	LEVEL -
A.- HUMIDITY (% dry matter).	80 %	65 %
B.- INOCULUM (lactic bacteria strain).	yes	no
C.- $Ca(OH)_2$ (control of initial pH = 6.5)	yes	no
D.- TEMPERATURE	37°C	20°C
E.- MOLASSES (fermentable carbohidrate).	yes (10g)	no
F.- UREA (C/N).	yes (10g)	no
G.- CELLULOSE (fermentable carbohidrate/ raw fiber).	yes (10g)	no

TABLE 3

CONDITIONS OF DIFFERENT FACTORS FOR EACH EIGHT TREATMENT.

TREATMENT / FACTOR	1	2	3	4	5	6	7	8
ENRICHED MEAL	100 g	100 g	100 g	100 g	100 g	100 g	100 g	100 g
UREA	0	10 g	10 g	0	0	0	10 g	10 g
CELLULOSE	10 g	0	10 g	0	10 g	0	0	10 g
MOLASSES	10 g	10 g	0	0	0	10 g	0	10 g
INOCULUM	0	0	10 ml	10 ml	0	0	10 ml	10 ml
$Ca(OH)_2$	2 g	0	0	2 g	2 g	0	0	2 g
H_2O	241 ml	480 ml	213 ml	430 ml	223 ml	440 ml	196 ml	550 ml
INCUBATION TEMPERATURE	20°C	20°C	20°C	20°C	37°C	37°C	37°C	37°C