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Production of lipase (S) by Some Yeasts :

II. Effect of Some Carbohydrates and Nitrogenous
Compounds

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with 2 Figures

Maximum lipase production by all experimented yeasts was obtained in presence of 0.5 % yeast extract only. All tested nitrogenous compounds failed to do so. When the medium was amended with xylose or starch, maximum production of lipase per unit of medium was achieved by Rhodotorula rubra, Candida utilis and C. guilliermondii, while Sucrose and maltose showed the same effect on using Debaryomyces hansenii.

Introduction :

It has been suggested that certain lipases are adaptive in nature and their production is stimulated by the presence of specific substrates. In other words, the elaboration of the enzymes depends upon preferential utilization of certain substrates by the organisms (Cutchins et al., 1952).

Sometimes, lipase production depends essentially on the organism itself as mentioned by Naylor et al., 1930.

Chopra & Chander (1983) observed that lipase production by Syncephalastrum racemosum was subjected to catabolite repression when certain nitrogen and carbon sources were used in the growth medium.

Ammonium ions increased lipase production when used as nitrogen source by Torulopsis sp. (Dasouki, 1976), while they completely inhibited the synthesis of the same enzyme by Pseudomonas fluorescens (Cutchins et al., 1952). On the other hand a small amount of lipase was obtained by Thermoactinomyces vulgaris (Elwan et al., 1978).

Different carbon sources were found to be optimal for lipase biosynthesis. Yasuhida et al. (1973, 1978) used 2 % glucose and 2 % olive oil for the production of lipase by Candida paralipolytica. Soybean meal and bran stimulated lipase formation when added separately to C. paralipolytica 739 cultures as sole carbon source (Vecozola & Luka, 1979).

The present investigation aimed to study the effect of some carbon and nitrogen sources on lipase production by certain yeasts.

Material and Methods :

Test organisms and medium:-

The same medium as well as the four yeasts, Candida utilis, C. guilliermondii, Rhodotorula rubra and

Debaryomyces hansenii used in a previous investigation (El Gammal & Rhizk, 1984) were tested.

Preparation of cell-Free culture filtrates and estimation of growth :

Five ml of the "growth culture filtrates six day old were centrifuged at 3000-4000 rpm for 10 minutes and the supernatant was stored at 0°C.

The enzyme was assayed as previously described by El-Gammal & Rhizk (1984). Yeast cells were washed three times with distilled water, dried at 90°C in an electric oven for 16 h and then reweighed. Growth was expressed in terms of mg dry wt/ml broth.

Effect of nutrients :

The effect of the following groups of nutrients was examined. Various sugars (sucrose, lactose, maltose, arabinose and xylose), starch and glycerol were separately incorporated in the growth medium to study their effect on enzyme production. They were added in such amounts that provide the carbon weight located in 0.5 % of glucose.

As nitrogen sources, peptone was separately replaced by the following nitrogen compounds, NaNO_3 , casein hydrolysate, beef extract and urea in 0.5 % concentration(W/V).

Results and Discussion :

Results recorded in Table (1) and represented in Fig.(1) show that lipase production by the four different yeasts was greatly affected by the kind of carbon source. For D. hansenii, sucrose was the most favourable for lipase production; maltose showed the same effect followed by lactose and arabinose.

However, xylose, starch and glycerol slightly enhanced lipase production. In case of the anascosporogenous yeasts, C.utilis, C.guilliermondii and R.rubra either xylose or starch induced lipase productivity. All the other used sugars decreased lipase production. These results are in agreement with those obtained by Mohaved (1984) who stated that the most suitable sugars for the induction of lipase production by Aspergillus sydowi were sucrose and D(+) xylose since their presence in the mineral salts production medium gave the highest yield of lipase. The noticed result that D.hansenii favoured sucrose as the best carbon source for lipase production is in accordance with what has been noticed by Ammar & McDaniel (1979). They found that 0.5% sucrose was the best sugar and concentration for inducing lipase formation by Bacillus stearothermophilus.

Interestingly, there was a relatively limited stimulation when 1 % starch was added to the cultural medium of D.hansenii and C.utilis. However, for the other yeasts; C.guilliermondii and R.rubra 100% and 30% induction respectively was observed. Although starch inhibited lipase production when it was supplied to Aspergillus sydowi either separately or in combination with oil in the mineral salts medium (Mohawed 1983), yet starch was used in the production medium of certain yeast lipase e.g Torulopsis ernobii (Yoshida et al., 1968).

The failure of glycerol to induce lipase production by the yeasts under investigations may be explained by the suggestion of Temmisto (1933) that certain lipolytic species are unable to hydrolyze fat when they are grown on glycerol - fat agar due to the inactivation of the lipase by acid developed in glycerol fermentation .

Table (2) and Fig(2) represent the effect of different nitrogen sources on lipase production by the four experimented yeasts. One notes that omission of peptone from the growth medium, using yeast extract only stimulated maximum lipase production by all the tested yeasts, though a concentration of 2 % peptone gave maximum lipase production by many microorganisms (Tsujisaka

et al., 1973, Akhtar, 1979; Chander et al., 1980).

Our results are also contradictory to those obtained by Hosono & Tokita (1970) who found enhancement of lipase production by Candida mycoderma on using peptone.

However, the present results agree with those obtained by Vecozola & Luka (1979) who used 1-2 % yeast autolysate for the production of lipase by Candida paralytolytica. Also, Chander et al., (1980) found that addition of 0.5% (W/V) yeast extract to the medium had a stimulatory effect on the synthesis of lipase by Aspergillus wentii.

The present results show that incorporation of yeast extract in the medium was essential for the optimum production of the enzyme. Peptone gave increased growth than all other nitrogenous tested compounds, but there was a corresponding marked reduction in lipase synthesis when expressed per unit of growth. Also lipase reduction was obtained with casein in case of C.utilis, casein and urea in case of C.guilliermondii and all other tested nitrogenous sources in case of R.rubra and D.hansenii.

The data in Table (2) represent good evidence that lipase production by C.utilis, C.guilliermondii, R.rubra and D.hansenii was subjected to catabolite repression when certain nitrogenous compounds were incorporated in the

growth medium. This is in agreement with Chopra et al. (1983) who experimented with Syncephalastrum racemosum using groundnut protein, Soybean meal, milk casein and wheat bran as sources of nitrogen.

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Table (1) : Effect of some sugars and other carbon compounds on growth and lipase production by *Candida utilis*, *G. Sullitermondii*, *Rhodotorula rubra* and *Debaryomyces hansenii*.

Carbon Source	<i>C. utilis</i>			<i>G. Sullitermondii</i>			<i>R. rubra</i>			<i>D. hansenii</i>		
	biomass mg dry wt/ml broth	lipase/ ml broth	lipase/ mg dry wt.	biomass mg dry wt/ml broth	lipase/ ml broth	lipase/ mg dry wt.	biomass mg dry wt/ml broth	lipase/ ml broth	lipase/ mg dry wt.	biomass mg dry wt/ml broth	lipase/ ml broth	lipase/ mg dry wt.
Lactose	0.90	3.9	22.1	0.70	2.2	15.9	0.88	3.9	29.2	0.69	4.6	34.3
Sucrose	0.92	3.9	21.6	0.96	2.2	11.6	0.90	3.9	22.1	0.69	7.5	39.5
Arabinose	0.89	3.4	19.4	0.90	2.2	12.4	0.90	3.4	19.2	0.98	4.6	23.8
Maltose	0.94	2.2	11.9	0.62	2.2	18.0	0.90	3.4	19.2	0.90	7.5	42.2
Xylose	1.85	7.6	20.5	1.89	7.6	20.1	1.91	10.2	26.8	1.83	3.9	10.8
Sterch	1.85	6.6	17.2	1.97	7.6	19.2	1.90	8.7	22.9	1.92	3.9	10.3
Glycerol	0.96	3.4	18.0	1.11	2.2	10.0	0.89	3.9	22.3	0.84	3.9	23.6
No sugar	1.86	5.8	23.4	1.84	3.0	9.4	1.89	5.0	20.0	1.85	3.4	9.3

Table (2): Effect of some nitrogen sources on growth and lipase production by Candida utilis, C. Guilliermondii, Rhodotorula rubra and Debryomyces hansenii.

Nitrogen Source	C. utilis			C. Guilliermondii			R. rubra			D. hansenii		
	biomass mg dry wt./ml broth	lipase/ mg dry wt.	lipase/ mg	biomass mg dry wt./ml broth	lipase/ mg dry wt.	lipase/ mg	biomass mg dry wt./ml broth	lipase/ mg dry wt.	lipase/ mg	biomass mg dry wt./ml broth	lipase/ mg dry wt.	lipase/ mg
Yeast extract	0.348	10.9	31.5	0.220	5.4	24.9	0.208	8.7	41.9	0.208	3.9	19.1
Na NO ₃	0.200	10.9	54.7	0.218	4.6	21.4	0.356	3.9	11.1	0.352	3.4	9.8
Casein	0.366	10.9	29.8	0.214	3.9	18.6	0.352	6.3	17.9	0.962	-	-
Beef extract	0.204	10.9	53.6	0.212	4.6	22.0	0.374	7.9	21.2	0.358	3.9	11.1
Urea	0.240	10.9	45.5	0.204	2.9	14.4	0.356	5.4	15.4	0.208	3.4	16.6
Peptone	0.372	6.3	23.4	0.368	3.4	9.4	0.378	6.8	20.0	0.370	3.4	9.3

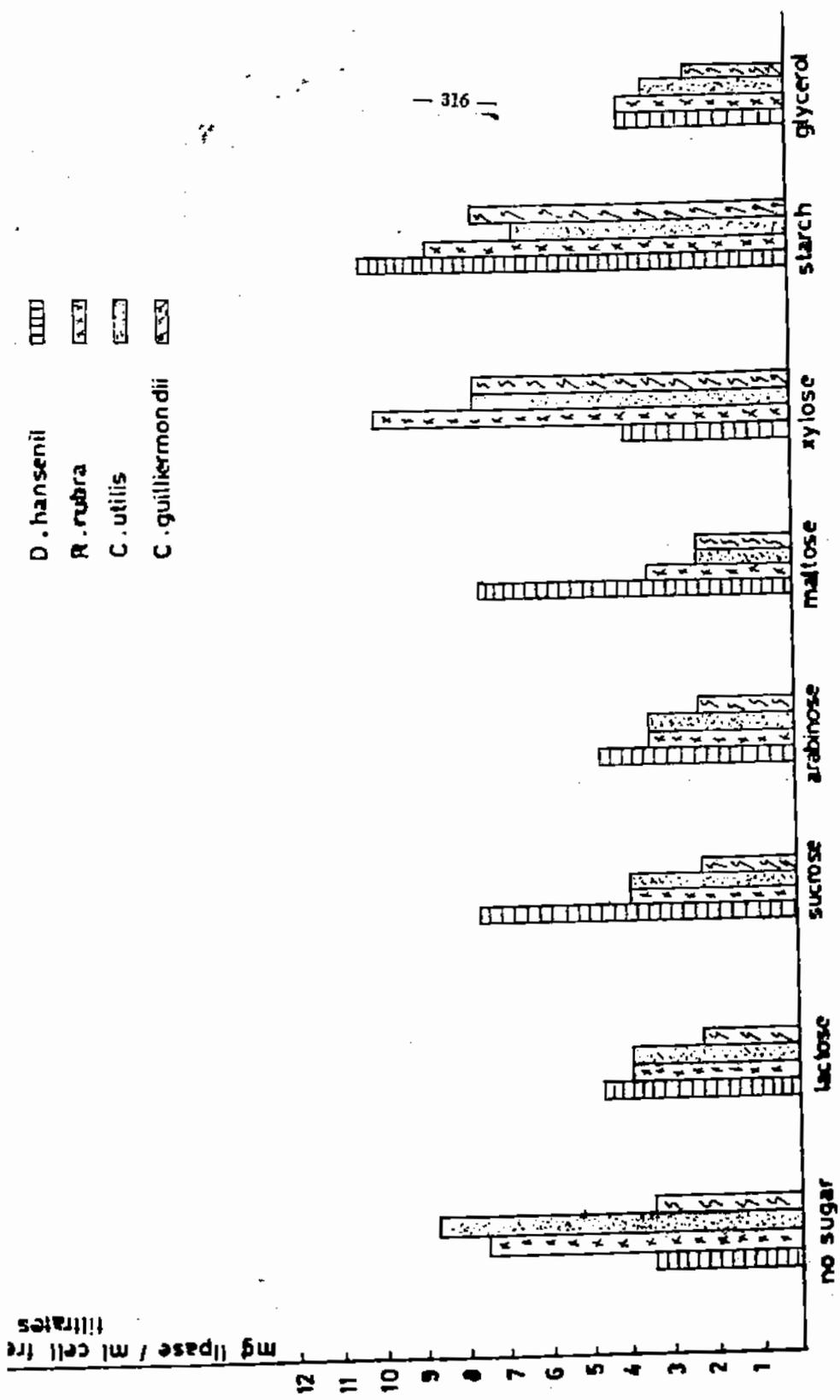


Fig. (1) : Effect of Some Sugars on Lipase Production by C. utilis, C. guilliermondii, R. rubra and D. hanseni.

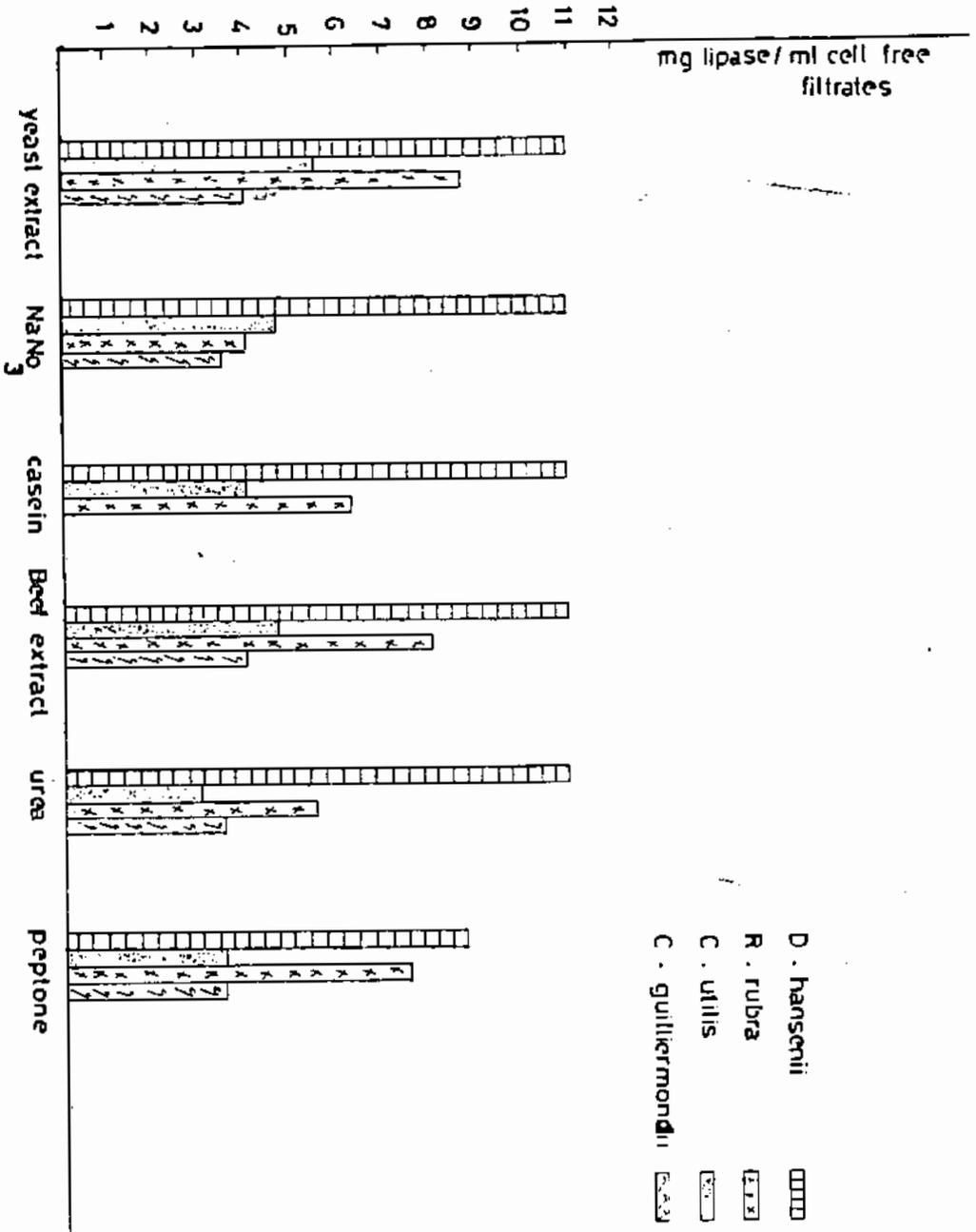


Fig. (2) : Effect of Some Nitrogenous supplements on Lipase production by *C. utilis*, *C. guilliermondii*, *R. rubra* and *D. hansenii*.

قسم النبات - كلية البنات - جامعة عين شمس

انتاجية

انزيم اللييز بواسطة بعض الخمائر

٢- اثر بعض المواد الكربوهيدراتية والنتروجينية

موسم محمد عبد العزيز الجمال ، منى أحق رزق

أظهرت الخمائر المختبرة كلها أعلى إنتاجية لانزيم اللييز

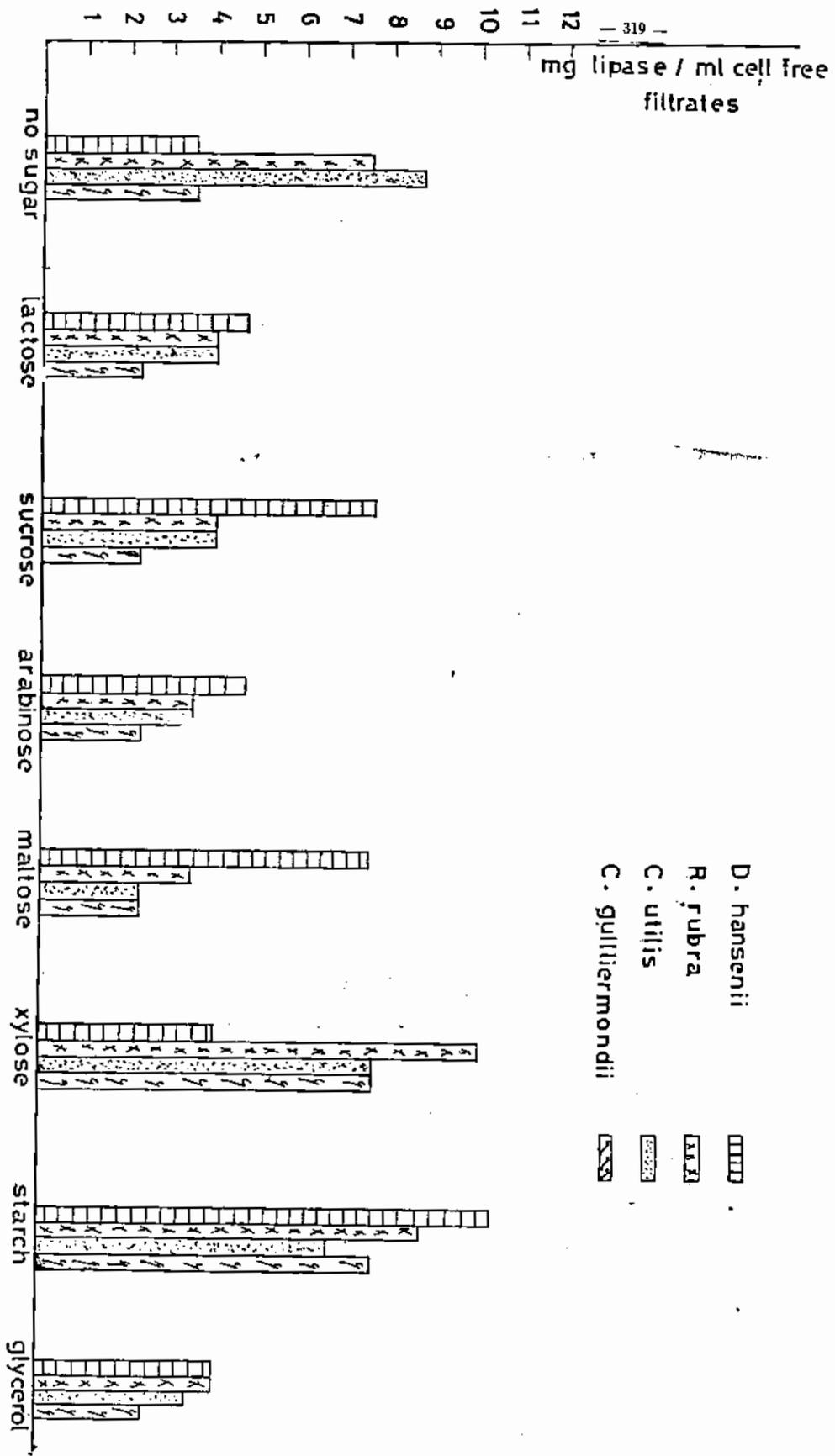
بإضافة ٥% مستخلص الخميرة لبيئة النمو بينما اختلفت جميع المواد

النتروجينية الأخرى في إعطاء أعلى إنتاجية . ونتج عن إضافة سكر

الزيلوز أو النشا إلى بيئة النمو أكبر كمية من الإنزيم بواسطة كل من

خميرتي رودوثوريولا روسا وكانديدا ييثيلس بينما نتج نفس التأثير في

حالة دياروسيس هانغياى بإضافة السكر أو المالتوز .



D. hansenii

R. rubra

C. utilis

C. guilliermondii

Fig. (1) : Effect of some Sugars on Lipase Production by *C. utilis*, *C. guilliermondii*, *R. rubra* and *D. hansenii*.

