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Intra- and extra-cellular lipid production by yeasts

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it is difficult to explain why part of it is not converted to higher fatty acids. It could be suggested that the yeasts which produce intra- and extra-cellular lipids contain an enzyme which preferentially esterifies acetic acid with the C₆- and C₅-polyalcohols. As the resulting esters of these polyalcohols with acetic and higher fatty acids were found to be the constituents of the extra-cellular lipids, it might be assumed that the cell membrane is highly permeable for these particular esters. More evidence as to the validity of the above-mentioned hypothesis may be obtained by the use of cell free extracts. Experiments on this subject will be carried out in the near future.

The importance of both intra- and extra-cellular lipids for the longevity of the yeast cells was demonstrated with five yeast species. They all gave similar results. In the absence of available carbon and nitrogen compounds in the nutrient medium both lipids were used up by the cells within one or two months. During the period of lipid consumption the number of viable cells did not vary much, but the dry weight of yeast decreased considerably. When the lipids had disappeared the viability of the cells began to decrease; their dry weight remained nearly constant, however. This demonstrates that the dead cells were not utilized by the living ones. The results of the latter experiments show that the lipids may be considered as a normal reserve food, a conclusion which is in agreement with the statement of STEINER (1957b).

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SUMMARY

1. A study was made of the formation of lipids by yeast cells. Two of the six species tested viz. *Lipomyces starkeyi* and *Rhodotorula gracilis* produced only intra-cellular lipids, the other four viz. *Rhodotorula graminis*, two strains of *Rhodotorula glutinis* and *Candida bogoriensis* in addition formed extra-cellular lipids.

2. In shake cultures at 29°C yeast growth and lipid production were found to be nearly proportional to glucose consumption. Formation of lipids started when the available nitrogen of the medium had been used up, but in *Lipomyces starkeyi* and in *Candida bogoriensis* it started earlier. The maximum CoA content of the cells (100–120 units per g dry yeast) was attained at the beginning of lipid formation. Optimal yeast growth and lipid synthesis were obtained at a pH value between 4.5 and 6.0, *Lipomyces starkeyi*, however, had its optimal pH between 2.2–5.0. Increasing amounts of nitrogen in the nutrient medium gave a marked decrease in lipid content of the dry yeast of *Rhodotorula gracilis* and *Rhodotorula graminis*, but with *Lipomyces starkeyi* this effect was much less pronounced.

3. The main fatty acids in the intra-cellular lipids of the six yeast species investigated were always palmitic and oleic acids. Linoleic acid occurred to the extent of about 10% in the lipids of the *Rhodotorula* species. In *Lipomyces starkeyi* this value was lower, but in *Candida bogoriensis* it amounted to nearly 20%. The incubation temperature had only a slight effect on the composition of the intra-cellular lipids.

4. The fatty acids in the extra-cellular lipids differed considerably from those of the intra-cellular lipids, mainly owing to the presence of acetic acid, which formed about 50% of the total amount of fatty acids. Palmitic and oleic acids occurred also in considerable amounts in the excreted lipids, but part of the higher fatty acids could not be identified. A cultivation temperature of 18°C gave a higher content of acetic acid in the extra-cellular lipids than a temperature of 29°C, but the effect on the higher fatty acids in these lipids was quite arbitrary. In the culture solution of *Candida bogoriensis* crystals of unknown structure were sometimes formed, particularly at 18°C.

5. The influence of the pH of the nutrient medium on amount and composition of the lipids was studied with *Rhodotorula graminis*. Optimal formation of lipids occurred at pH 5–6.5. A rise in pH from 4.25 to 7.8 gave an increase in the palmitic acid and a decrease in the oleic acid content of the intra-cellular lipids. In the extra-cellular lipids the amount of acetic acid dropped with increasing pH.

6. The effect of various C-sources on the production and composition of lipids was studied with *Lipomyces starkeyi*. Glycerol and succinic acid gave a similar fatty acid composition to glucose. Palmitic, oleic and linoleic acids, however, gave fat which contained much larger amounts of the respective acids supplied.

7. Glycerol was the alcoholic component of the lipids of *Lipomyces starkeyi*, *Rhodotorula gracilis* and *Candida bogoriensis*. In *Rhodotorula graminis* and *Rhodotorula glutinis* glycerol and sorbitol were the alcoholic components of the intra-cellular lipids, whereas the extra-cellular lipids contained sorbitol and a trace of a C₅-polyalcohol.

8. Both intra- and extra-cellular lipids were used as a substrate by the yeasts when they were incubated at 29°C without C- and N-sources.

SAMENVATTING

1. Een onderzoek werd verricht over de vorming van vet en vetachtige stoffen door gisten. Dit onderzoek werd uitgevoerd met zes gistsoorten. Twee van deze zes, nl. *Lipomyces starkeyi* en *Rhodotorula gracilis*, vormden alleen intra-cellulaire lipiden, terwijl *Rhodotorula graminis*, twee stammen van *Rhodotorula glutinis* en *Candida bogoriensis* bovendien extra-cellulaire lipiden produceerden.

2. In schudcultures bij 29° bleken gistgroei en productie van lipiden bij alle onderzochte gistsoorten praktisch evenredig te zijn aan het glucoseverbruik. De vorming van lipiden begon wanneer de beschikbare stikstof in het medium was opgebruikt, maar bij *Lipomyces starkeyi* en *Candida bogoriensis* eerder. Het maximum gehalte van de gistcellen aan CoA (100–120 eenheden per gram droge gist) werd bereikt bij het begin van de lipiden vorming. Optimale gistgroei en vorming der lipiden vonden plaats bij een pH waarde tussen 4.5 en 6.0. Bij *Lipomyces starkeyi* echter lag het optimum tussen pH 2.2–5.0. Toenemende

hoeveelheden stikstof in het cultuur medium gaven bij *Rhodotorula gracilis* en *Rhodotorula graminis* een duidelijke daling van de hoeveelheid intra-cellulaire lipiden; bij *Lipomyces starkeyi* was dit effect veel geringer.

3. De belangrijkste vetzuren in de intra-cellulaire lipiden van de zes onderzochte gistsoorten waren steeds palmitine- en oliezuur. Linolzuur kwam in de lipiden van de *Rhodotorula*-soorten in hoeveelheden van ongeveer 10% voor. Bij *Lipomyces starkeyi* was deze waarde lager, maar bij *Candida bogoriensis* bedroeg hij bijna 20%. De temperatuur, waarbij de gisten gekweekt werden, had slechts een geringe invloed op de samenstelling van de intra-cellulaire lipiden.

4. De vetzuren van de extra-cellulaire lipiden verschilden aanzienlijk van die der intra-cellulaire lipiden; dit werd hoofdzakelijk veroorzaakt door de aanwezigheid van azijnzuur (50% van de totale hoeveelheid vetzuren). Palmitine- en oliezuur kwamen ook in aanzienlijke hoeveelheden in de uitgescheiden lipiden voor; een deel van de hogere vetzuren kon echter nog niet worden geïdentificeerd. Bij een kweektemperatuur van 18° was het gehalte aan azijnzuur in de extra-cellulaire lipiden hoger dan bij een temperatuur van 29°C, maar de invloed op het gehalte aan hogere vetzuren in deze lipiden was tamelijk onregelmatig. *Candida bogoriensis* vormde soms kristallen van onbekende samenstelling in het voedingsmedium, in het bijzonder bij 18°C.

5. De invloed van de pH van de voedingsoplossing op hoeveelheid en samenstelling van de lipiden werd onderzocht bij *Rhodotorula graminis*. Maximale lipidenvorming trad op bij pH 5-6.5. Een stijging van de pH van 4.25 tot 7.8 gaf bij de intra-cellulaire lipiden een toename in het gehalte aan palmitinezuur en een afname in dat aan oliezuur. In de extra-cellulaire lipiden nam de hoeveelheid azijnzuur af met stijgende pH.

6. De invloed van verschillende C-bronnen op de productie en de samenstelling van de lipiden werd onderzocht bij *Lipomyces starkeyi*. Glycerol en barnsteenzuur gaven een zelfde vetzuursamenstelling als glucose. Palmitine-, olie- en linolzuur, echter, gaven lipiden die grote hoeveelheden van de respectievelijk toegevoegde zuren bevatten.

7. In de lipiden van *Lipomyces starkeyi*, *Rhodotorula gracilis* en *Candida bogoriensis* was glycerol de alcoholische component. Bij *Rhodotorula graminis* en *Rhodotorula glutinis* waren glycerol en sorbitol de alcoholische componenten van de intra-cellulaire lipiden, terwijl de extra-cellulaire lipiden sorbitol en een spoorje van een C₅-polyalcohol bleken te bevatten.

8. Zowel intra- als extra-cellulaire lipiden werden als substraat gebruikt door de gistcellen, wanneer deze zonder C- en N-bron bij 29°C werden gekweekt.

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