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Publication Date:
1979

Permanent Link:
https://doi.org/10.3929/ethz-a-000173846 →

PIGMENTED STREPTOCOCCI

A CHEMOSTAT STUDY OF PIGMENT PRODUCTION IN RUMEN ISOLATES OF *Streptococcus bovis*

A dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH

for the degree of Doctor of Natural Sciences

presented by

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1979

5. SUMMARY

7 of 8 strains of S. bovis, including all 6 rumen isolates studied, produced pigment on agar media if CO_2 gas was added to the anaerobic jar. Bicarbonate or Tween 80 added to the medium did not replace the CO_2 requirement for pigment production. Pigment was not produced when the gas overlay in an anaerobic, glucose limited chemostat culture of S. bovis 2B was changed from 100% CO_2 to 5% CO_2 - 95% N_2. This dependence on gaseous CO_2 probably explains the failure of other authors to list pigment as a stable characteristic of S. bovis rumen strains.

Pigment production (measured with a simple spectrophotometric assay) and lactic acid yield of S. bovis 2B increased with D and culture pH in the anaerobic glucose limited chemostat, while acetate and ethanol production decreased proportionally. Pigment increased one generation after a D step-up or a glucose pulse, but not after a starch pulse.

With ammonia limiting, the culture continued to use all the glucose in the medium and cell yield on glucose decreased 40%. Division irregularities characteristic of "unbalanced growth" and cell lysis were observed in thin sections of the cells. Cystine was not used as a reserve ammonia source. Data with glucose limitation indicated a cystine yield of 12.9 g DCW per g cystine, but S. bovis 2B preferentially used inorganic sulfate. Slow growing ammonia or glucose limited cells had thicker cell walls and less bound pigment than faster growing organisms.

Cell clumping was observed during the transition from batch to continuous culture, after pH disturbances, and during washout.

Previously excreted pigment returned to the surface of the clumped cells. Clumps seen with scanning electron microscopy were closely packed and round in comparison with single cells from the complex medium inoculum. Freeze fracture photos of clumps showed a striking difference in the distribution of the particles on the inner membrane from that in fast growing chemostat or batch culture cells. It is suggested that clumping serves to cut the area for transport in times when nutrient oversupply may hinder balanced growth.

Pigment isolated from chemostat supernatants by acid precipitation was in a poorly soluble complex with protein and lipoteichoic acid like material. Precipitated pigment was extremely stable to acid and its visible spectrum altered with the polarity of the solvent it was redissolved in. Methanol extraction of the complex gave a fluorescent yellow compound that appeared to be a peptide. Fluorescence disappeared when PAN, a specific Cu chelator, was added.

Although some S. bovis strains take up haematin from the growth medium, the pigment from defined medium contains no iron and no spectral evidence for cytochromes was found. Ascorbate altered the pigment spectrum. Pigment is not a reserve substrate and is not involved in aerobic metabolism or sulfate reduction. It could be a detoxification product, a bacterial lectin, or a metal ion chelator.

9. ZUSAMMENFASSUNG

Die Farbstoffproduktion (FP) in S. bovis ist von der Kulturbedingungen abhängig. Alle sechs untersuchten Pansenisolate produzieren in Petrischalenversuchen Pigment, falls die anaerobische Atmosphäre mit CO_2 angereichert ist. In glukoselimitierten, anaeroben Chemostaten blieb die FP von S. bovis 2B aus, falls die 100% CO_2 Begasung auf 5% CO_2 / 95% N_2 herabgesetzt wurde. Die FP nimmt mit steigender Verdünnungsrate (D) zu. Laktatproduktion nimmt mit steigender D und fallendem pH (D konstant) zu, Acetat- und Aethanolproduktion nehmen jedoch ab. Die FP nahm ca. 1 Generation nach Erhöhung der Glukosezugaberate zu. Zellen von D=0.06 h^{-1} hatten dickere Zellwände und weniger gebundenes Pigment als Zellen von D=0.33 h^{-1}.

In ammoniumlimitierten anaeroben Chemostaten war die Zellausbeute gegenüber Glukoselimitation um 40% reduziert. Unregelmässigkeiten der Zellteilung ("unbalanced" Wachstum) wurde beobachtet. Cystin wurde nicht als zusätzliche N-Quelle verwendet.

Beim Uebergang von Batch zur kontinuierlichen Kultur, beim Auswaschen, sowie nach pH-Störungen traten Zellagglomerationen auf. Die Zellen in Klumpenverband sind im Vergleich zu Einzelzellen rund und enggepackt, und haben eine unterschiedliche Partikelseggregation auf der Innenseite der Membrane.

Das aus dem Kulturüberstand durch Ansäuren gefällte Pigment ist zusammen mit protein- und lipoteichonsäureahnlichem Material gebunden. Die Cu-Konzentration korreliert mit OD_{400} und Fluoreszenz. Die vorläufige Charakterisierung des Pigments zeigte, dass es sich um ein Metallchelat oder ein Lektin handeln dürfte.