LYCOPENE ACCUMULATION IN PIGMENTED RADIO-RESISTANT *MICROCOCCI* GROWN IN PRESENCE OF NICOTINE

Norman F. LEWIS and Umesh S. KUMTA
Biochemistry and Food Technology Division
Bhabha Atomic Research Centre,
Trombay, Bombay 85, India

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1. Introduction

It is fairly well established [1] that geranyl pyrophosphate is the immediate precursor for carotenoid biosynthesis in microorganisms. The first carotenoid synthesized is phytoene which is converted into lycopene through a series of dehydrogenation steps via phytofluene ζ-carotene and neurosporene. Moreover, there is evidence that both the acyclic carotenoids lycopene and neurosporene are substrates for the cyclization reaction leading to β-carotene synthesis [1].

Recent work by Howes and Batra [2] on the effects of the alkaloid nicotine on carotenoid biosynthesis in two *Mycobacterium* species indicated that nicotine inhibits the cyclization reaction resulting in cellular accumulation of the acyclic carotenoid lycopene. It was further shown that removal of nicotine resulted in conversion of accumulated lycopene to β-carotene, the major polyene of these cells. An identical effect has been reported by Goodwin [1] to occur in cells of *Phycomyces blakesleeanus* and a *Flavobacterium* species. The purpose of this communication is to present evidence that a similar phenomenon occurs in cells of pigmented *Micrococci* which can tolerate exceptionally high doses of both gamma and UV radiation [3, 4] – the pigments of these *Micrococci* are predominantly keto-carotenoids.

2. Methods and materials

Micrococcus radiophilus isolated by Lewis [5] from irradiated Bombay duck (*Harpodon nehereus*), and *Micrococcus radiodurans*, were taken from our stock culture collection for these studies. The organisms were grown in TGYM medium according to conditions described by Lewis [3], and also in TGYM medium containing the alkaloid nicotine (10^{-2} M). Cellular pigment was extracted from harvested cells using $CHCl_3$:CH_3OH (2:1) – extractions were repeated (usually thrice) till cells were visibly decolorized. The pooled extracts were saponified for 1 hr at 40° with methanolic KOH; the unsaponifiable pigments were extracted in diethyl ether, washed free of alkali, evaporated to dryness under N_2 and stored at −10° till they were used for TLC analysis. TLC plates were run in a solvent system containing C_6H_6:CH_3OH:CH_3COOH (87:11:2) [6]. Individual carotenoids which separated as distinct bands were scraped off the plates and their spectra determined in a Beckman spectrophotometer Model DB-G. Each of these spectra exhibited similar absorption characteristics – a broad range (λ_{max} 480 nm). The spectral characteristics of the total pigment fraction obtained from TGYM cells and nicotine-grown cells were also examined.

3. Results and discussion

The spectra of pigments of TGYM cells were typical of keto-carotenoids possessing a broad range with a maxima at 480 nm (fig. 1) as compared with pigments of the nicotine-grown cells whose spectra showed fine structure with maxima at 455, 480 and 510 nm (fig. 2). The major pigment of nicotine-

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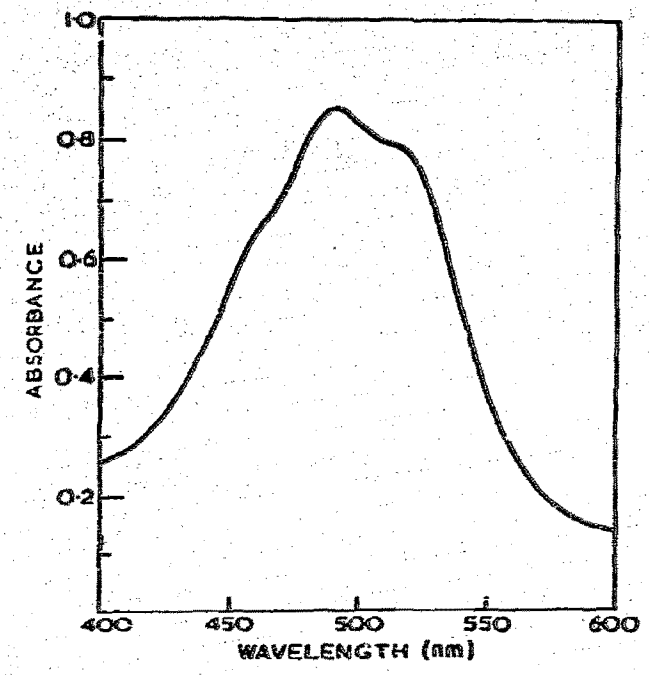

Fig. 1. The absorption spectra of the total pigment fraction of cultures of *M. radiophilus* grown in TGYM medium. Pigment extracts of cultures of *M. radiodurans* showed similar spectra.

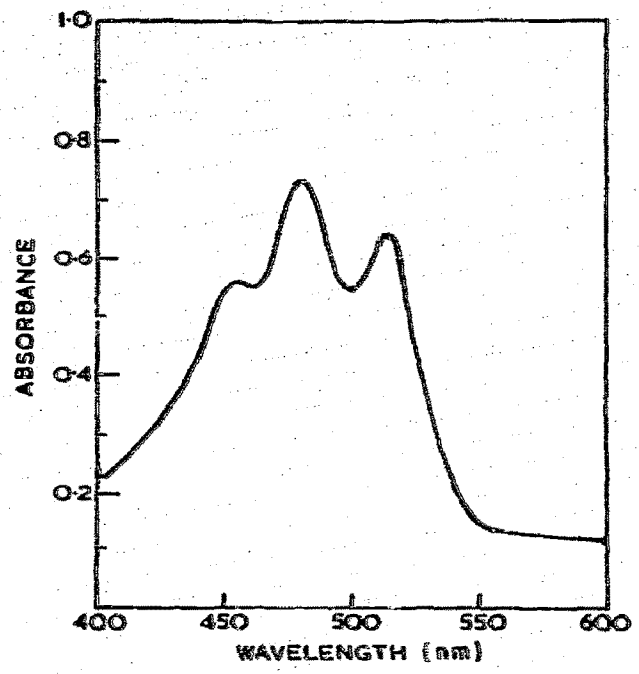

Fig. 2. The absorption spectra of the total pigment fraction of cultures of *M. radiophilus* grown in TGYM medium containing nicotine (10^{-2} M). Pigment extracts of cultures of *M. radiodurans* showed similar spectra.

grown cells was identified as lycopene using pure lycopene as standard. Examination of pigment extract of TGYM cells after reduction with $NaBH_4$ showed a change in spectra from a broad absorption spectrum (λ_{max} 480 nm) to one of fine structure (λ_{max} 450, 480, 512 nm) indicative of the reduction of an allylic keto group to a hydroxyl group [7]. No cyclic carotenoids could be determined in the pigment extracts of nicotine-grown cells. These findings indicate that nicotine inhibits the sequence of carotenoid biosynthesis in these *Micrococci* at the lycopene → β-carotene stage resulting in accumulation of lycopene in the cells. Moreover, since nicotine was present during growth of these cells and not added to grown cells as in the investigations of Howes and Batra [2] and Goodwin [1], it appears that nicotine inhibits the biosynthesis of enzyme(s) involved in the cyclization reaction.

Lewis et al. [8] observed that TGYM cells of *M. radiophilus* were inactivated by gamma radiation with concomitant destruction of cellular pigment (keto carotenoids) as compared with nicotine-grown cells of *M. radiophilus* whose pigment (lycopene) was unaffected even after a dose of 3.0 Mrad which brought about a six log reduction in cell count; similar findings were also observed with cells of *M. radiodurans* by these workers [8], who concluded that cellular pigments do not play any significant role in the radio-resistance of these *Micrococci*.

Cells of *M. radiophilus* and *M. radiodurans* cultured in the presence of nicotine were centrifuged and washed free of nicotine with phosphate buffer. The washed cells were suspended in flasks containing fresh TGYM medium, phosphate buffer (pH 7.0), physiological saline, or 0.5% glucose and incubated at 30° up to 72 hr. Pigment extracts from cells harvested only from flasks containing TGYM were now found to predominate in keto-carotenoids with concomitant disappearance of lycopene. Pigment extracts of cells harvested from flasks containing buffer, saline or glucose, were devoid of keto-carotenoids — the major polyene present in these extracts was lycopene. This finding indicates that on removal of nicotine, lycopene is converted to β-carotene which is

subsequently oxidized to the keto-carotenoids, and that these reactions occur only in complete growth medium. This finding corroborates that nicotine must be inhibiting the synthesis of enzyme(s) involved in the cyclization of lycopene to β-carotene, which synthesis occurs only in complete growth medium (TGYM medium).

The distinct steps in the carotenoid biosynthesis occurring in these radio-resistant *Micrococci* are not known; moreover, individual cellular carotenoids have not been characterized. Hence, it is not known precisely at what stage nicotine exerts an inhibitory influence. However, since lycopene has been identified as the major pigment in nicotine-grown cells which are devoid of the cyclic carotenoids, it must act as a potent inhibitor of cyclohexylidene ring formation in the biosynthetic sequence of carotenoids in these organisms. Further, since on removal of nicotine, lycopene is converted to the keto-carotenoids, lycopene is the substrate for the cyclization reaction in these *Micrococci* species.

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