glucosyl), 3.85 (*br*, sugar protons), 6.42 (1H, *d*, $J = 2$ Hz, H-6), 6.80 (1H, *d*, $J = 2.5$ Hz, H-8), 7.67 (2H, *dd*, $J = 2.5$, 8.5 Hz, H-2′–H-6′); 6.95 (1H, *d*, $J = 8.5$ Hz, H-5′).

On acid hydrolysis the compound gave ombuin and chromatography of the aq. layer on Whatman No. 1 paper in EtOAc–Py–H_2O (12:5:4) and EtOAc–*i*-PrOH–H_2O (3:1:1) showed glucose and rhamnose. On methylation (Me_2SO_4–K_2CO_3) and hydrolysis, 5,7,3′,4′-tetramethylquercetin was identified by mp and co-chromatography with an authentic sample.

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EFFECT OF NICOTINE ON CAROTENOGENESIS IN EXTREMELY HALOPHILIC BACTERIA

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Key Word Index—*Halobacterium halobium*; *Sarcina litoralis*; *Amoebobacter morrhuae*; extremely halophilic bacteria; pigmented strains; morphologically different; nicotine; carotenoids; bacterioruberins.

Nicotine, first introduced by Howes and Batra in 1970 as a bioregulator of carotenogenesis in mycobacteria [1, 2], has since been reported to affect carotenogenesis in photosynthetic and non-photosynthetic bacteria [3–6], fungi [7, 8] and higher plants [9].

Recently we have found that nicotine had a pronounced effect on carotenogenesis, particularly of the C_{50} pigments in *Halobacterium cutirubrum* [10]. In this report we examine the effect of nicotine on carotenogenesis in three representative and morphologically different genera of extreme halophiles, i.e. *H. halobium* (rod shape), *Sarcina litoralis* (a halococcus) and *Amoebobacter morrhuae* (a highly pleomorphic form of the genus *Halobacterium*). Our results show that nicotine is a potent regulator of carotenogenesis in extreme halophiles, irrespective of their morphological differences.

When the above strains of extreme halophiles were grown in the presence of increasing concentrations of nicotine, the formation of bacterioruberin and monoanhydrobacterioruberin was completely inhibited by 1.0 mM nicotine (Table 1). Concomitantly, large increases in levels of lycopene and bisanhydrobacterioruberin were observed. The maximum accumulation of these two compounds occurred at 3 mM nicotine for *H. halobium* and *S. litoralis* and at 6 mM nicotine for *A. morrhuae*. The total carotenoid content of the three strains at the optimal concentrations of nicotine was usually 24–31% higher than that of control cultures (Table 1). Such a stimulatory effect of nicotine on total carotenoid production has also been observed in photosynthetic bacteria [4]. It is also noteworthy that growth of the above organisms is not inhibited in up to 6 mM nicotine, but at higher concentrations it is drastically reduced.

The results presented in this paper indicate that nicotine is a potent inhibitor of the C_{50} bacterioruberin and monoanhydrobacterioruberin and also of β-carotene in the genus *Halococcus* (e.g. *Sarcina litoralis*), in the rod-shaped halophiles of genus *Halobacterium* (e.g. *H. halobium*) and in *Amoebobacter morrhuae*, a highly pleomorphic halobacterium, as was reported previously for *Halobacterium cutirubrum* [10]. The present findings are also consistent with the view presented previously [10] that the C_{50} carotenoids may be formed from a C_{40} carotene, probably lycopene.

EXPERIMENTAL

Cultures of the above microorganisms were grown aerobically for 5 days at 37° in 1 l. batches of standard complex medium for halophiles in 4 l. shake flasks in an incubator shaker as described previously [11]. Appropriate amounts of nicotine were added, aseptically, at the time of inoculation as described elsewhere [10]. Cells were harvested, washed and estimated for protein content by the method of Lowry *et al.* [12]. Total lipids were extracted by the Bligh–Dyer procedure [13] and neutral lipids were separated by acetone precipitation of the total lipids as described previously [10, 11]. Individual carotenoids were purified by applying the acetone-soluble lipids to Si gel G TLC plates and developing in $CHCl_3$–MeOH (93:7) for bacterio-

Table 1. Effect of various concentrations of nicotine on carotenoid composition in extremely halophilic bacteria*

Nicotine (mM)	Growth, g. protein per l. culture	Carotenoid					
		β-Carotene	Lycopene	Bisanhydrobacterioruberin	Monoanhydrobacterioruberin	Bacterioruberin	Total carotenoids
				(μg/g cell protein)			
			Halobacterium halobium, 34020				
0	1.5	7	T	6	43	180	236
1	1.47	0	200	40	T	T	240
3	1.21	0	260	50	T	T	310
6	1.18	0	250	50	T	T	300
9	0.89	0	150	20	T	T	170
12	0.30	0	50	10	T	T	60
15	0.10	0	20	5	T	T	25
			Sarcina litoralis, 16006				
0	0.48	2	T	3	20	100	125
1	0.43	0	100	20	T	T	120
3	0.43	0	130	30	T	T	160
6	0.30	0	110	20	T	T	130
9	0.28	0	75	10	T	T	85
12	0.16	0	30	10	T	T	40
15	0.09	0	8	7	T	T	15
			Amoebobacter morrhuae, 51001				
0	1.2	3	2	8	70	400	483
1	1.11	0	415	80	T	T	495
3	1.11	0	430	110	T	T	540
6	1.02	0	450	150	T	T	600
9	0.91	0	350	75	T	T	425
12	0.74	0	210	60	T	T	270
15	0.46	0	75	50	T	T	125

* For details of microorganisms and their National Research Council of Canada culture collection number, see ref. [11].
T = traces.

ruberin and monoanhydrobacterioruberin; in $CHCl_3$–Et_2O (98:2) for bisanhydrobacterioruberin; and in petrol–Et_2O (98:2) for lycopene and β-carotene. The purified carotenoids were quantified by their UV–vis spectra using extinction values reported previously [10, 11].

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