Transformation of *Micrococcus lysodeikticus* by Various Members of the Family Micrococcaceae

By W. E. KLOOS

*Department of Genetics,
North Carolina State University, Raleigh,
North Carolina 27607, U.S.A.*

(Accepted for publication 29 August 1969)

SUMMARY

Various strains originally designated as members of the genera *Micrococcus*, *Sarcina*, and *Staphylococcus* were able to transform adenine, histidine, and tryptophan auxotrophs of *Micrococcus lysodeikticus* to prototrophy at relatively high frequencies. These included certain strains of *M. flavocyaneus*, *M. flavus*, *M. luteus*, *M. lysodeikticus*, *M. sodonensis*, *Sarcina flava*, *S. lutea*, *S. subflava*, *Staphylococcus afermentans* and *St. flavocyaneus*. Many of the above strains were adenine auxotrophs and produced low frequencies of prototrophic recombinants when crossed with the adenine-dependent *M. lysodeikticus* strain ISU. Prototrophic strains of *M. conglomeratus*, *M. radiodurans*, *M. roseus*, *M. rubens*, *M. varians*, *St. aureus* and *St. epidermidis* failed to transform *M. lysodeikticus* in these studies. Prototrophic pigment mutants of *M. lysodeikticus* and *S. lutea* were able to doubly transform yellow auxotrophs of *M. lysodeikticus* to prototrophy and donor colour.

INTRODUCTION

The taxonomic status of the 'species' *Micrococcus lysodeikticus*, isolated by Fleming (1922), has been under investigation for a number of years. One significant step toward classification was made when Evans, Bradford & Niven (1955) proposed including this organism in the genus *Micrococcus* and separating it from the staphylococci on the basis of its relation to oxygen (obligate aerobe) in a standardized complex medium containing glucose. As a result of numerous physiological and biochemical studies, Kocur & Martinec (1962, 1963, 1965) and Rosypal & Kocur (1963) have suggested that strains of *M. lysodeikticus* be included together with certain strains of *M. cyaneus*, *M. flavocyaneus*, *M. flavus*, *M. luteus*, *M. sodonensis*, *Sarcina aurantiaca*, *S. citrea*, *S. flava*, *S. lutea*, *S. marginata*, *S. pelagia*, *S. subflava*, *S. variabilis*, *Staphylococcus afermentans*, and *St. flavocyaneus* into a single species, *M. luteus*. Baird-Parker (1963, 1965) also combined *M. lysodeikticus* with representatives of the above organisms into a single taxonomic group, *Micrococcus* subgroup 7. More recent studies by Rosypal, Rosypalova & Horejs (1966), based on DNA base composition and Adansonian analysis, have shown that most of the strains classified as *M. luteus* by Kocur & Martinec possessed high coefficients of similarity (S value) and had approximately similar GC contents in their DNA. However, they proposed subdividing these micrococci on the basis of DNA base ratios: group 1 (*M. flavocyaneus*, *M. flavus*, *M. lysodeikticus*, *M. sodonensis*, *S. citrea*, *S. flava*, *S. lutea*, *S. marginata*, *S. pelagia*, *S.*

subflava, *S. variabilis*, *St. fermentans*, and *St. flavocyaneus*) having 70·8 to 73·3% GC; group 2 (*S. aurantiaca* and several other micrococci) having 67·5 to 69·5% GC; and group 3 (*M. luteus* strain ATCC 398 and *M. cyaneus*) having 65·8 to 67·0% GC.

Preliminary studies on transformation in *Micrococcus lysodeikticus* (Kloos &

Table 1. Transformation of nutritional markers in *Micrococcus lysodeikticus* by various members of the family Micrococcaceae

Donor species*	Strain	Source†	Nutritional classification including mutant or auxotrophic genotype‡	Prototrophs/10 ⁶ colony-forming units resulting in crosses with <i>M. lysodeikticus</i> strain ISU auxotrophs§		
				<i>ade</i>	<i>trpE</i> 16	<i>hisD</i> 61
Micrococcus						
<i>M. candidans</i>	ATCC 8456	1	—	< 1	< 1	< 1
<i>M. citreus</i>	ATCC 395	1	—	< 1	< 1	< 1
<i>M. conglomeratus</i>	ATCC 401	1	+	< 1	< 1	< 1
<i>M. flavocyaneus</i>	ATCC 8673	2	<i>ade</i>	26	888	283
<i>M. flavocyaneus</i>	CCM 622	3	<i>ade</i>	10	530	126
<i>M. flavocyaneus</i>	CCM 851	3	<i>ade</i>	12	572	131
			<i>ade</i> ⁺ -1	297	1279	348
<i>M. flavocyaneus</i>	CCM 853	3	<i>ade</i>	16	613	172
<i>M. flavus</i>	ATCC 400	1	<i>ade</i>	9	238	82
<i>M. flavus</i>	ATCC 10240	2	+	192	470	174
<i>M. luteus</i>	ATCC 398	1,4	—	< 1	< 1	< 1
<i>M. luteus</i>	CCM 105	3	<i>ade</i>	11	1150	271
			<i>ade</i> ⁺ -1	340	1279	230
<i>M. luteus</i>	CCM 308	3	+	220	1080	210
<i>M. luteus</i>	CCM 355	3	+	234	760	179
<i>M. luteus</i>	CCM 370	3	<i>ade</i>	8	852	140
<i>M. luteus</i>	CCM 408	3	<i>ade</i>	23	1088	96
			<i>ade</i> ⁺ -1	414	1236	74
<i>M. luteus</i>	CCM 1423	3	+	696	665	263
<i>M. luteus</i>	CCM 1672	3	<i>ade</i>	32	1390	260
			<i>ade</i> ⁺ -1	374	1240	188
<i>M. lysodeikticus</i>	ISU	5	<i>ade</i>	< 1	1176	478
			<i>ade</i> ⁺ -1	346	1899	232
			<i>ade</i> ⁺ -1 <i>trpE</i> 16	563	< 1	190
			<i>ade</i> ⁺ -1 <i>hisD</i> 61	548	1260	< 1
<i>M. lysodeikticus</i>	PU	5	<i>ade</i>	34	2394	227
			<i>ade</i> ⁺ -8	374	2126	307
<i>M. lysodeikticus</i>	UM	5	<i>ade</i>	11	1211	203
			<i>ade</i> ⁺ -1	261	1013	180
<i>M. lysodeikticus</i>	WRU	5	<i>ade</i>	9	1812	198
			<i>ade</i> ⁺ -1	381	1762	186
<i>M. lysodeikticus</i>	ATCC 4698	1	<i>ade</i>	9	2046	350
<i>M. lysodeikticus</i>	ATCC 15801	4	<i>ade</i>	16	784	102
			<i>ade</i> ⁺ -4	338	627	118
<i>M. lysodeikticus</i>	CCM 1335	3	+	276	967	151
<i>M. radiodurans</i>	ATCC 13939	6	+	< 1	< 1	< 1
<i>M. roseus</i>	ATCC 412	1	+	< 1	< 1	< 1
<i>M. roseus</i>	ATCC 416	1	+	< 1	< 1	< 1
<i>M. roseus</i>	ATCC 516	1	+	< 1	< 1	< 1
<i>M. roseus</i>	ATCC 9815	1	+	< 1	< 1	< 1
<i>M. roseus</i>	B-P 94	7	+	< 1	< 1	< 1
<i>M. roseus</i>	B-P 97	7	—	< 1	< 1	< 1
<i>M. rubens</i>	ATCC 186	1	+	< 1	< 1	< 1
<i>M. sodonensis</i>	ATCC 11880	1	+	264	935	217
<i>M. varians</i>	ATCC 399	1	+	< 1	< 1	< 1

Table 1 *cont.*

Donor species*	Strain	Source†	Nutritional classification including mutant or auxotrophic genotype‡	Prototrophs/10 ⁶ colony- forming units resulting in crosses with <i>M.</i> <i>lysodeikticus</i> strain ISU auxotrophs§		
				<i>ade</i>	<i>trpE</i> 16	<i>hisD</i> 61
Sarcina						
<i>S. aurantiaca</i>	ATCC 146	4	—	< 1	< 1	< 1
<i>S. flava</i>	ATCC 540	4	<i>ade</i>	24	972	162
<i>S. lutea</i>	ATCC 272	3	+	235	708	359
<i>S. lutea</i>	ATCC 381	4	<i>ade</i>	17	512	163
<i>S. lutea</i>	ATCC 382	2	(—)	500	638	190
<i>S. lutea</i>	ATCC 533	8	+	325	1479	161
<i>S. subflava</i>	ATCC 7468	4	+	240	855	320
<i>S. ureae</i>	ATCC 6473	4	—	< 1	< 1	< 1
<i>S. ureae</i>	NCDO 1548	9	—	< 1	< 1	< 1
Staphylococcus						
<i>St. afermentans</i>	ATCC 15307	4	+	806	2568	204
<i>St. aureus</i>	655	10	(+)	< 1	< 1	< 1
<i>St. aureus</i>	83	10	(+)	< 1	< 1	< 1
<i>St. epidermidis</i>	B-P 287	7	(+)	< 1	< 1	< 1
<i>St. epidermidis</i>	B-P 2	7	(+)	< 1	< 1	< 1
<i>St. epidermidis</i>	B-P 12	7	(+)	< 1	< 1	< 1
<i>St. epidermidis</i>	B-P 21	7	(+)	< 1	< 1	< 1
<i>St. epidermidis</i>	B-P 31	7	(+)	< 1	< 1	< 1
<i>St. flavocyaneus</i>	CCM 247	3	<i>ade</i>	18	641	221

* Strains which were originally designated *Micrococcus flavocyaneus* (CCM622, CCM851, CCM853), *M. lysodeikticus* (CCM1335), *Sarcina lutea* (ATCC272), and *Staphylococcus flavocyaneus* (CCM247) were received from M. Kocur as strains of *M. luteus* (Kocur & Martinec, 1962; 1963).

† 1—J. B. Evans, North Carolina State Univ., Raleigh, N.C., U.S.A.; 2—E. R. Kennedy, Catholic Univ., Washington, D.C., U.S.A.; 3—M. Kocur, Czechoslovak Collection of Microorganisms, J. E. Purkyne Univ., Brno, CSSR; 4—American Type Culture Collection, Rockville, Maryland, U.S.A.; 5—E. A. Grula, Oklahoma State Univ., Stillwater, Okla., U.S.A.; 6—A. H. Schein, Univ. of Vermont, Burlington, Vermont, U.S.A.; 7—A. C. Baird-Parker, Unilever Research Laboratory, Bedford, England; 8—T. Tornabene, Colorado State Univ., Fort Collins, Colo., U.S.A.; 9—E. I. Garvie, National Collection of Dairy Organisms, Univ. of Reading, Reading, England; 10—P. A. Pattee, Iowa State Univ., Ames, Iowa, U.S.A.

‡ *ade* = Adenine requirement (growth stimulated by adenine, adenosine, inosine, or hypoxanthine); *ade*⁺ = adenine-independent mutant, notation followed by arabic numeral to indicate order of isolation, e.g. *ade*⁺-1; *trpE* 16 = L-tryptophan requirement (growth stimulated by anthranilic acid, indole, or L-tryptophan); *hisD* 61 = L-histidine requirement (growth stimulated by L-histidine but not by L-histidinol); — = no detectable growth (colonies) on defined agar, phenotype unclassified; (—) = no detectable growth on defined agar, but genotype is believed to be *ape*⁺ *trpE*⁺ *hisD*⁺ based on transformation data; (+) = no detectable growth on defined agar of Kloos & Schultes (1969), but prototrophic phenotype classified on defined agar for staphylococci of Kloos & Pattee (1965); + = prototroph.

§ Data represent the average of two separate DNA preparations. Two crosses were made from each preparation and each cross plated on duplicate plates. Recipient controls without DNA produced < 1 prototroph/10⁶ colony-forming units.

Schultes, 1969) have shown that *Sarcina lutea* strain ATCC272 was able to transform adenine auxotrophs of certain *M. lysodeikticus* strains to prototrophy, providing evidence of close genetic relationship. Extending transformation studies between *M. lysodeikticus* and other members of the family Micrococcaceae would offer the possibility of examining genetic exchange among these organisms and would broaden our understanding of a genetic definition of *Micrococcus* species, *i.e.* those micrococci

that can potentially contribute to, or share in, a common gene pool (Marmur, Falkow & Mandel, 1963). In this investigation, various Micrococcaceae have been tested for transforming activity of nutritional markers in crosses with the high transformation frequency *M. lysodeikticus* strain ISU (Kloos, 1969; Kloos & Schultes, 1969). In addition, preliminary studies on transformation of pigment markers is reported.

METHODS

Bacterial strains. Donor strains selected for this study were originally designated as members of the genera *Micrococcus*, *Sarcina* and *Staphylococcus* and were, in particular, those sensitive to either lysozyme or lysostaphin (Table 1).

Cultures received by this laboratory were checked for purity and to some extent proper identification by (1) colony morphology and pigment production on a complex agar medium (P agar) (Naylor & Burgi, 1956) incubated at 26 and 34° and (2) microscopic examination of Gram-stained cells. Each strain was also tested for prototrophy, with respect to adenine, L-tryptophan, and L-histidine, by examining colony formation on a defined agar medium (Kloos & Schultes, 1969) incubated at 26 and 34°. Strains which failed to produce detectable colonies or colonies of less than 0.1 mm. diameter by 8 days were further tested for adenine, L-tryptophan, and/or L-histidine auxotrophy on defined agar media supplemented with each metabolite (20 µg./ml.) singly or in combinations.

Table 2. *Double transformation of pigment and nutritional markers in Micrococcus lysodeikticus*

Prototrophic donor species	Strain	Pigment genotype*	Donor pigment-prototrophs/10 ⁸ colony-forming units resulting in crosses with <i>M. lysodeikticus</i> strain ISU yellow-auxotrophs†		
			<i>pig-y ade</i>	<i>pig-y trpE16</i>	<i>pig-y hisD61</i>
<i>M. lysodeikticus</i>	ISU	<i>pig-w</i>	1.2	6.7	1.0
		<i>pig-p</i>	1.9	7.3	1.5
		<i>pig-o</i>	0.9	5.0	0.8
<i>M. lysodeikticus</i>	ATCC 15801	<i>pig-p</i>	1.0	4.1	0.5
<i>Sarcina lutea</i>	ATCC 272	<i>pig-w</i>	0.6	2.4	0.4
		<i>pig-p</i>	1.0	4.7	0.4
		<i>pig-o</i>	0.7	1.9	0.5

* *pig-w* = white; *pig-p* = pink; *pig-o* = orange.

† Data represent the average of two separate DNA preparations. Two crosses were made from each preparation and each cross plated on ten plates. Recipient controls without DNA or DNA isolated from yellow strains produced < 0.1 donor pigment-prototroph/10⁸ colony-forming units.

Spontaneous adenine-independent mutants were isolated from certain adenine-dependent strains and used as donors. Several different pigment mutants of *Micrococcus lysodeikticus* strain ISU and *Sarcina lutea* strain ATCC 272 were obtained by treatment of yellow strains with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) (300 µg./ml.) (Adelberg, Mandel & Chen, 1965) and were also used as donors (Table 2).

Recipient strains included the adenine-dependent *Micrococcus lysodeikticus* strain ISU and a tryptophan and histidine auxotroph derived from a spontaneous adenine-independent mutant (*ade*⁺-1) of this strain by treatment with NTG (Table 1). The tryptophan and histidine auxotrophs were biochemically characterized by growth

stimulation studies and chromatographic analysis of de-repressed culture supernatants (Kloos & Pattee, 1965; Rose & Kloos, 1969).

Procedure for DNA isolation. *Staphylococcus aureus* and *St. epidermidis* strains were grown in tryptic soy broth (Difco) at 37° for 18 hr. All other donor strains were grown in a glucose+yeast extract broth (Rosypalova, Bohacek & Rosypal, 1966) at 30° for 18 to 24 hr. Cultures were shaken during incubation with a Burrell Wrist-Action Shaker (Burrell Corporation, Pittsburgh, Pennsylvania) at a setting of two giving 324 shakes/min. through an arc of 3°. The yield in 100 ml. of broth was about 1 to 3 g. wet packed cells. After growth, cells were harvested by centrifugation and washed twice with a NaCl-EDTA solution: 0.15M-NaCl+0.10M-ethylenediamine-tetraacetic acid (EDTA), pH 8.0. DNA was isolated by the procedure of Marmur (1961) with the modification of Saito & Miura (1963) using T1 ribonuclease (RNase) (Sankyo Company, Ltd., Tokyo, Japan) as well as bovine pancreatic RNase (Calbiochem, Los Angeles, California). The duration of lysozyme (Calbiochem) treatment varied from 1 hr to as long as 12 hr for more resistant strains. Lysostaphin (kindly supplied by Dr W. A. Zygmunt, Mead Johnson Research Centre, Evansville, Indiana) was used in place of lysozyme to lyse *St. aureus* strains. The final DNA fibres were sterilized in 75% ethanol for 2 hr and then dispersed into a $\frac{1}{10}$ dilution of sterile standard saline solution (SS: 0.1M-NaCl, pH 7.0) which was adjusted to SS as soon as dispersion was completed. DNA preparations were used within 2 days of isolation and were stored at 4°. DNA concentration was determined by the diphenylamine reaction of Dische (1955).

Procedure for transformation. An 18 hr P agar slope culture of the recipient strain was suspended in 1 ml. saline and diluted 1/100 in saline. Aliquots of 0.1 ml. (about 5×10^6 colony-forming units) from the diluted suspension were added to duplicate tubes containing 1 ml. defined broth (Kloos, 1969) supplemented with 10 µg./ml. adenine (strain ISU *ade*), 10 µg./ml. L-histidine (strain ISU *ade*⁺-1 *hisD* 61), or 15 µg./ml. L-tryptophan (strain ISU *ade*⁺-1 *trpE* 16). Mixtures were shaken in a 34° water bath with a Burrell Wrist-Action Shaker at a setting of four giving 324 shakes/min. through an arc of 6° for 12 hr (strain ISU *ade*⁺-1 *hisD* 61) or 24 hr (strains ISU *ade* and ISU *ade*⁺-1 *trpE* 16). After growth, cocci (about $1-3 \times 10^8$ colony-forming units/ml.) were centrifuged and resuspended in 1 ml. transformation buffer: 0.05M-tris (hydroxymethyl) aminomethane (tris)+0.01M-SrCl₂+0.5% monosodium glutamate, pH 7.0. DNA (10 µg. in 0.03 to 0.8 ml. SS) was added to each tube and the mixtures shaken in a 30° water bath at a setting of four for 1 hr. Exposure to DNA was terminated by the addition of deoxyribonuclease (5 µg./ml.) (Worthington Biochemical Corporation, Freehold, New Jersey) and 0.005M-MgSO₄ with incubation at 37° for 15 min. After treatment, cocci were centrifuged and resuspended in 1 ml. saline. Samples of 0.1 ml. were taken from the saline suspension, a 10⁻¹ and 10⁻² dilution in saline and spread on duplicate defined agar plates. Prototrophs were scored after incubation at 34° for 72 hr. Additional plates were prepared and incubated at 26° for 72 hr in crosses where the donor strain had a more optimum growth at 26° than at 34°. Plates failing to show significant numbers of prototrophic transformants were incubated for an additional 5 days. In crosses where pigment mutants were used as donors, pigment-prototrophs (double transformants) were scored after incubation for 6 days to allow for pronounced pigment production.

RESULTS

Nutritional characterization of donor strains. Many of the donor strains tested were able to produce relatively large colonies (0.5 to 2.0 mm. diam. in 96 hr) on the defined agar medium used in transformation experiments (Table 1). These were considered prototrophic with respect to adenine, L-histidine, and L-tryptophan as these nutrients were omitted from the medium.

Some of the strains tested proved to be adenine auxotrophs and were markedly stimulated by either adenine, adenosine, inosine, or hypoxanthine. For example, auxotrophs that showed no detectable growth or colonies less than 0.1 mm. diam. (leaky strains) on defined agar media produced colonies of 0.5 to 1.6 mm. diam. in 96 hr on defined agar media supplemented with 20 µg. of adenosine per ml. Adenine auxotrophy has been reported previously in strains of *Micrococcus lysodeikticus* (Grula, Luk & Chu, 1961; Kloos & Schultes, 1969); however, it was interesting to note that other strains having different species designations were also auxotrophic. Cultures of *M. luteus* strains CCM105, CCM408, CCM1672 and *M. flavocyaneus* strain CCM851 were received containing both adenine-independent and adenine-dependent cells in ratios (Ade⁺/Ade) of 1/9, 1/116, 1/1890 and 1/36, respectively.

Transformation of nutritional markers. DNA isolated from certain strains of *Micrococcus flavocyaneus*, *M. flavus*, *M. luteus*, *M. lysodeikticus*, *M. sodonensis*, *Sarcina flava*, *S. lutea*, *S. subflava*, *Staphylococcus afermentans* and *St. flavocyaneus* was able to transform auxotrophs of *M. lysodeikticus* to prototrophy (Table 1). In all instances, the above strains transformed *M. lysodeikticus* strain ISU at near or equal to homologous, intra-strain frequencies. Variation between DNA preparations from the same strain generally did not exceed 60% of the mean value.

Adenine-dependent donor strains produced low but significant frequencies of prototrophic transformants when crossed with adenine-dependent *Micrococcus lysodeikticus* strain ISU. These results would suggest that adenine auxotrophy, in these strains, was the effect of mutation at sites different from the one present in the recipient strain. The very low transformation values obtained may be evidence of mutation in the same adenine locus, though consideration of mutation in different adjacent or closely linked loci, at this point, cannot be excluded. Studies are being pursued to determine the specific enzyme(s) affected in these auxotrophs.

Double transformation of pigment and nutritional markers. It would be of interest to extend genetic studies and test transformation of additional markers with those strains capable of transforming *Micrococcus lysodeikticus* to prototrophy. One class of mutants that can be identified readily and are produced in high frequencies is pigment mutants. For example, treatment of *M. lysodeikticus* strain ISU (ade⁺-1) with NTG, to obtain auxotrophs used as recipients in this study, resulted in the production of about 2% pigment mutant, sectorial colonies. These included various intensities of yellow, white, pink, orange, chartreuse (similar to the colour of *Sarcina lutea* strain ATCC272), and a water-soluble yellow class which dispersed yellow pigment in the surrounding culture medium. Several of these mutants were selected and used as donors in crosses with yellow auxotrophic strains (Table 2). Pigment transformants were scored selectively as double transformants with prototrophy. In this preliminary study, pigment mutants of *S. lutea* strain ATCC272 were isolated and used as donors with recipients of *M. lysodeikticus*. The range of different colours produced by treat-

ment with NTG was similar to that found with *M. lysodeikticus*. As shown in Table 2, all mutants tested were able to transform yellow auxotrophs of *M. lysodeikticus* strain ISU to the donor colour. The fraction of double pigment-prototroph transformants/single prototroph transformants for each recipient auxotroph was similar and was approximately 1/360. Additional studies were conducted which showed an increase in the fraction of double transformants/single transformants as the DNA concentration was increased up to saturation levels (Kloos, 1969). Collectively, these results would suggest that the pigment markers are not linked to DNA fragments containing the adenine (*ade*), histidine (*hisD*), and/or tryptophan (*trpE*) loci.

DISCUSSION

Genetic exchange has been shown to be useful in measuring homology between various bacteria and is usually parallel to the results obtained with nucleic acid hybridization (Dubnau, Smith, Morell & Marmur, 1965; Marmur *et al.* 1963). The demonstration of genetic exchange between *Micrococcus lysodeikticus* and certain strains designated as species of the genera *Micrococcus*, *Sarcina*, and *Staphylococcus* implies close genetic relationships among these organisms.

Transformation with such a large array of strains bearing different species and generic designations seems, at first glance, to be most unusual. In particular, nutritional markers are usually transformed only among very closely related organisms (Dubnau *et al.* 1965). These results, however, are in close agreement with the suggestions of others based on other criteria, classifying these strains as members of a single species, *Micrococcus luteus*. All strains participating in genetic exchange had very similar GC ratios, S values, and occurred in *Micrococcus* subgroup-1a of the classification of Rosypal *et al.* (1966).

Sarcina aurantiaca and *Micrococcus luteus* strain ATCC 398, previously considered by Kocur & Martinec (1962) to be *M. luteus* strains, failed to produce prototrophic transformants when crossed with *M. lysodeikticus*. These strains had distinctly lower GC ratios and, according to the above classification, were placed in subgroups 2c and 3b, respectively. *Micrococcus luteus* strain ATCC 398 also has a different menaquinone pattern than that shown for strains in subgroup 1a (Jeffries *et al.* 1968). It should be noted that these strains failed to grow on the defined medium used in transformation experiments and, therefore, any interpretation of negative results should be taken with some caution. Prototrophy with respect to adenine, L-tryptophan, and L-histidine is uncertain in these strains.

The failure of *Micrococcus candicans*, *M. citreus*, *M. conglomeratus*, *M. roseus*, *M. rubens* and *M. varians* to transform *M. lysodeikticus* was not surprising, as these organisms differ significantly in certain physiological and biochemical features and/or GC ratio (Baird-Parker, 1965; Baddiley, Brock, Davison & Partridge, 1968; Bohacek, Kocur & Martinec, 1967; Jeffries *et al.*, 1968; Rosypal *et al.* 1966). Transformation was not expected by strains of *Staphylococcus aureus* or *St. epidermidis* either, as members of the genus *Staphylococcus* as recommended by Evans *et al.* (1955) are facultative anaerobes and differ in numerous characteristics, including a very low GC ratio (32 to 35%) (Auletta & Kennedy, 1966; Baird-Parker, 1965; Garrity, Detrick & Kennedy, 1969; Jeffries *et al.* 1968). Two additional species designated *M. radiodurans* and *Sarcina ureae* failed to transform *M. lysodeikticus*. However, there is

strong evidence that these organisms should be omitted from the family Micrococcaceae (Baird-Parker, 1965; Herndon & Bott, 1969; Bohacek, Kocur & Martinec, 1967).

I am pleased to acknowledge Mrs Margaret Musselwhite and Mr R. Willis for their technical assistance. I also wish to thank Dr J. B. Evans for his encouragement and advice during this study.

This investigation was supported by the Public Health Service Grant AI 08255-01 from the National Institute of Allergy and Infectious Diseases.

This is paper number 2908 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh, North Carolina.

REFERENCES

- ADELBERG, E. A., MANDEL, M. & CHEN, G. C. C. (1965). Optimal conditions for mutagenesis by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine in *Escherichia coli* K12. *Biochem. biophys. Res. Commun.* **18**, 788.
- AULETTA, A. E. & KENNEDY, E. R. (1966). Deoxyribonucleic acid base composition of some members of the Micrococcaceae. *J. Bact.* **92**, 28.
- BADDILEY, J., BROCK, J. H., DAVISON, A. L. & PARTRIDGE, M. D. (1968). The wall composition of micrococci. *J. gen. Microbiol.* **54**, 393.
- BAIRD-PARKER, A. C. (1963). A classification of micrococci and staphylococci based on physiological and biochemical tests. *J. gen. Microbiol.* **30**, 409.
- BAIRD-PARKER, A. C. (1965). The classification of staphylococci and micrococci from world-wide sources. *J. gen. Microbiol.* **38**, 363.
- BOHACEK, J., KOCUR, M. & MARTINEC, T. (1967). DNA base composition and taxonomy of some micrococci. *J. gen. Microbiol.* **46**, 369.
- DISCHE, Z. (1955). Color reactions of nucleic acid components. In *The Nucleic Acids*. Ed. by E. Chargaff and J. N. Davidson, vol. 1, p. 285. New York and London: Academic Press.
- DUBNAU, D., SMITH, I., MORELL, P. & MARMUR, J. (1965). Gene conservation in *Bacillus* species. I. Conserved genetic and nucleic acid base sequence homologies. *Proc. natn. Acad. Sci. U.S.A.* **54**, 491.
- EVANS, J. B., BRADFORD, W. L. & NIVEN, C. F. (1955). Comments concerning the taxonomy of the genera *Micrococcus* and *Staphylococcus*. *Int. Bull. bact. Nomencl. Taxon.* **5**, 61.
- FLEMING, A. (1922). On a remarkable bacteriolytic element found in tissues and secretions. *Proc. R. Soc. Lond. B* **93**, 306.
- GARRITY, F. L., DETRICK, B. & KENNEDY, E. R. (1969). Deoxyribonucleic acid base composition in the taxonomy of *Staphylococcus*. *J. Bact.* **97**, 557.
- GRULA, E. A., LUK, S. K. & CHU, Y. C. (1961). Chemically defined medium for growth of *Micrococcus lysodeikticus*. *Can. J. Microbiol.* **7**, 27.
- HERNDON, S. E. & BOTT, K. F. (1969). Genetic relationship between *Sarcina ureae* and members of the genus *Bacillus*. *J. Bact.* **97**, 6.
- JEFFRIES, L., CAWTHORNE, M. A., HARRIS, M., COOK, B. & DIPLOCK, A. T. (1968). Menaquinone determination in the taxonomy of Micrococcaceae. *J. gen. Microbiol.* **54**, 365.
- KLOOS, W. E. (1969). Factors affecting transformation of *Micrococcus lysodeikticus*. *J. Bact.* **98**, 1397.
- KLOOS, W. E. & PATTEE, P. A. (1965). A biochemical characterization of histidine-dependent mutants of *Staphylococcus aureus*. *J. gen. Microbiol.* **39**, 185.
- KLOOS, W. E. & SCHULTES, L. M. (1969). Transformation in *Micrococcus lysodeikticus*. *J. gen. Microbiol.* **55**, 307.
- KOCUR, M. & MARTINEC, R. (1962). A taxonomic study of the genus *Micrococcus*. *Folia Fac. Sci. Natur. Univ. Purkynianae Brunensis* **3**, 1.
- KOCUR, M. & MARTINEC, R. (1963). The classification of some violet-pigmented micrococci. *J. gen. Microbiol.* **32**, 185.
- KOCUR, M. & MARTINEC, R. (1965). Some remarks on the classification of micrococci. *Int. Bull. bact. Nomencl. Taxon.* **15**, 113.

- MARMUR, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. molec. Biol.* **3**, 208.
- MARMUR, J., FALKOW, S. & MANDEL, M. (1963). New approaches to bacterial taxonomy. *A. Rev. Microbiol.* **17**, 329.
- NAYLOR, H. B. & BURGI, E. (1956). Observations on abortive infection of *Micrococcus lysodeikticus* with bacteriophage. *Virology* **2**, 577.
- ROSE, N. E. & KLOOS, W. E. (1969). Transforming system to study genetic relationships of tryptophan mutants of *Micrococcus lysodeikticus*. *Bact. Proc.* p. 59.
- ROSYPAL, S. & KOCUR, M. (1963). The taxonomic significance of the oxidation of carbon compounds by different strains of *Micrococcus luteus*. *Antonie van Leeuwenhoek* **29**, 313.
- ROSYPAL, S., ROSYPALOVA, A. & HOREJS, J. (1966). The classification of micrococci and staphylococci based on their DNA base composition and Adansonian analysis. *J. gen. Microbiol.* **44**, 281.
- ROSYPALOVA, A., BOHACEK, J. & ROSYPAL, S. (1966). Deoxyribonucleic acid base composition and taxonomy of violet-pigmented micrococci. *Antonie van Leeuwenhoek* **32**, 105.
- SAITO, H. & MIURA, K. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. biophys. Acta* **72**, 619.