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SHORT PAPERS

Transformation of *Sarcina flava* and *Micrococcus flavocyaneus**

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1. SUMMARY

Sarcina flava ATCC 540 (*ade*) and *Micrococcus flavocyaneus* ATCC 8673 (*ade*), two related micrococci, were transformed to prototrophy at frequencies as high as 0·02 % and 0·005 % of colony-forming units, respectively. Both of these organisms were transformed by selected prototrophic strains of *Micrococcus lysodeikticus*, *M. flavocyaneus*, *S. flava* and *Sarcina lutea*.

2. INTRODUCTION

Transformation in the genus *Micrococcus* has been limited to the species *Micrococcus lysodeikticus* (Kloos, 1968; Kloos & Schultes, 1969; Mahler & Grossman, 1968; Okubo & Nakayama, 1968). However, numerous strains designated as members of the genera *Micrococcus*, *Sarcina* and *Staphylococcus* can serve as donors in transformation with *M. lysodeikticus* (Kloos, 1969*a*, *b*). Those participating in genetic exchange had very similar DNA base composition (GC ratio), high coefficients of similarity (S value) in Adansonian analysis and were classified in Micrococcus subgroup 1a in the scheme of Rosypal, Rosypalova & Horejs (1966). Transformation has also been reported in *Micrococcus radiodurans* (Moseley & Setlow, 1968); however, suggestions have been made to classify this organism in a Gram-negative genus (Baird-Parker, 1965; Bohacek, Kocur & Martinec, 1967).

The present study was conducted to determine if various micrococci related to *M. lysodeikticus* could act as recipients in transformation.

3. MATERIALS AND METHODS

Bacterial strains

The bacterial strains selected for this study have been previously described (Kloos & Schultes, 1969; Kloos, 1969*b*) and are listed in Table 1.

Procedure for DNA isolation

Donor strains were grown in 100 ml peptone-yeast extract broth (Rosypalova, Bohacek & Rosypal, 1966) at 32 °C for 18–24 h. Cultures were shaken in a 1 l. flask on a rotary shaker (Fermentation Design, Inc., Allentown, Pennsylvania) at 350 rev/min. The yield of cocci was usually 1–3 g wet packed cells.

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DNA was isolated according to the procedure previously described (Kloos, 1969*b*). The duration of lysozyme treatment was usually 30 min–2 hr for most strains. *Sarcina flava* ATCC 540 required about 5–6 h lysozyme treatment for significant lysis to follow by the addition of sodium lauryl sulfate.

Table 1. *Bacterial strains*

Donor species	Strain	Genotype
Micrococcus lysodeikticus	ISU	*ade*
	ISU	*ade*$^+$*-1*
Micrococcus flavocyaneus	ATCC 8673	*ade*
	CCM 851	*ade*$^+$*-1*
Staphylococcus flavocyaneus	CCM 247	*ade*
Sarcina flava	ATCC 540	*ade*
	ATCC 540	*ade*$^+$*-1*
Sarcina lutea	ATCC 272	*ade*$^+$
	ATCC 533	*ade*$^+$
Recipient species		
Micrococcus lysodeikticus	ISU	*ade*
Micrococcus flavocyaneus	ATCC 8673	*ade*
	CCM 851	*ade*
	CCM 851	*trp-1*
	CCM 851	*his-1*
	CCM 853	*ade*
	CCM 622	*ade*
Micrococcus flavus	ATCC 400	*ade*
Micrococcus luteus (Kocur)	CCM 370	*ade*
Staphylococcus flavocyaneus	CCM 247	*ade*
	CCM 247	*trp-1*
	CCM 247	*his-1*
Sarcina flava	ATCC 540	*ade*
Sarcina lutea	ATCC 381	*ade*
	ATCC 272	*ade*
	ATCC 272	*trpE9*
	ATCC 272	*hisD1*

Procedure for transformation

Transformation was performed using a modification of the *M. lysodeikticus* tube method (Kloos, 1969*c*). An 18 h P agar (Naylor & Burgi, 1956) 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 tubes containing 1 ml defined broth supplemented with adenosine, L-histidine, or L-tryptophan (20 μg/ml). Mixtures were shaken in a 32° water bath with a Burrell Wrist-Action Shaker (Burrell Corporation, Pittsburgh, Pennsylvania) at a setting of 4 (324 shakes/min through an arc of 6°). The duration of incubation varied from 18–30 h depending upon the particular strain and was terminated when the cell density reached 1×10^8 colony-forming units/ml. After growth, cells were centrifuged and resuspended in 1 ml transformation buffer: 0·05 M-tris (hydroxymethyl) amino methane (Tris) + 0·01 M-$SrCl_2$, pH 7·0. DNA (10 μg) was added and the mixture was shaken in a 30° water bath at a setting of 4 for 1 h. Exposure to DNA was terminated by the addition of deoxyribonuclease (DNase) (5 μg/ml) (Worthington Biochemical Corporation, Freehold, New Jersey) and 0·005 M-$MgSO_4$. Cells were centrifuged and resuspended in 1 ml saline. Aliquots of 0·1 ml were taken from the saline suspension and from a 10^{-1} dilution in saline and spread

on duplicate defined agar plates (Kloos & Schultes, 1969). Prototrophs were scored after incubation at 32 °C for 48 h (*Sarcina lutea* ATCC 540) or 72 h (*Micrococcus flavocyaneus* ATCC 8673). Plates from crosses failing to show significant numbers of prototrophic transformants by 72 h were incubated for an additional 5 days.

4. RESULTS AND DISCUSSION

Various auxotrophic strains of micrococci related to *M. lysodeikticus* were tested for recipient competence in transformation. Results indicated that only two strains, *S. flava* ATCC 540 and *M. flavocyaneus* ATCC 8673, were transformed to prototrophy. Transformation of these strains was comparable, though somewhat reduced in frequency per colony-forming unit, to that found with *M. lysodeikticus* ISU (Table 2). Prototrophs of *S. flava* can be detected on defined agar media as early as 22–24 h. As this strain grows more rapidly than *M. flavocyaneus* or *M. lysodeikticus*, where prototrophs appear in about 36–40 h, it may have a particular advantage in genetic studies of nutritional characters.

The reciprocal transformation demonstrated in this study provides additional evidence of the close genetic relationship of these micrococci (Kloos, 1969*b*) and is consistent with the proposals of others (Kocur & Martinec, 1962; Baird-Parker, 1965; Rosypal *et al.* 1966) classifying these organisms into a single taxonomic group or species.

Auxotrophs of *M. flavocyaneus* CCM 851, CCM 853, CCM 622, *Staphylococcus flavocyaneus* CCM 247, *Micrococcus flavus* ATCC 400, *Micrococcus luteus* CCM 370, and *Sarcina lutea* ATCC 381, and ATCC 272 failed to be transformed to prototrophy with DNA from *M. lysodeikticus* ISU (*ade*$^+$*-1*, *M. flavocyaneus* CCM 851 (*ade*$^+$*-1*), *S. lutea* ATCC 272 (*ade*$^+$) or homologous DNA under the experimental conditions used.

Table 2. *Transformation of* Sarcina flava, Micrococcus flavocyaneus *and* Micrococcus lysodeikticus *adenine auxotrophs*

			Prototrophs/10^6 colony-forming units in crosses with *ade* recipients		
Donor species	Strain	Genotype	*Sarcina flava* ATCC 540	*Micrococcus flavocyaneus* ATCC 8673	*Micrococcus lysodeikticus* ISU
Micrococcus lysodeikticus	ISU	*ade*	4·1	< 0·01	0·1
	ISU	*ade*$^+$*-1*	73·0	39·0	365·5
Micrococcus flavocyaneus	ATCC 8673	*ade*	6·5	< 0·01	21·0
	CCM 851	*ade*$^+$*-1*	97·6	44·2	306·0
Staphylococcus flavocyaneus	CCM 247	*ade*	0·6	< 0·01	12·0
Sarcina flava	ATCC 540	*ade*	0·4	< 0·01	23·5
	ATCC 540	*ade*$^+$*-1*	191·4	52·8	320·6
Sarcina lutea	ATCC 272	*ade*$^+$	132·7	7·8	288·3
	ATCC 533	*ade*$^+$	122·2	6·9	316·5
Without DNA	—	—	0·2	< 0·01	0·1

Transformation was performed using those conditions shown to be optimal for *M. lysodeikticus*. However, it was considered to be of interest to test the effects of the divalent cations Mg, Ca, Ba or Sr on transformation in *S. flava* and *M. flavocyaneus*. Results

shown in Table 3 indicate that the relative efficiencies of these ions to promote transformation are essentially similar in both organisms and are comparable with that shown in *M. lysodeikticus* (Kloos, 1969*c*). As the transformation frequency is fairly low in *M. flavocyaneus*, the failure to obtain prototrophs with Mg^{2+} should be interpreted with some caution. Addition of DNase (5 μg/ml) + 0·005 M-$MgSO_4$ to recipient cells just prior to contact with DNA or to the DNA preparation resulted in the complete loss of prototrophic recombinants.

Table 3. *Effect of divalent cations on transformation*

Ion	Concentration (M)	Prototrophs/10^6 colony-forming units in crosses with *ade* recipients: *Sarcina flava* ATCC 540	Prototrophs/10^6 colony-forming units in crosses with *ade* recipients: *Micrococcus flavocyaneus* ATCC 8673
None	—	0·8	< 0·01
$MgSO_4$	10^{-3}	5·6	< 0·01
	10^{-2}	13·5	< 0·01
$CaCl_2$	10^{-3}	7·1	0·2
	10^{-2}	20·6	4·5
$BaCl_2$	10^{-3}	10·4	1·4
	10^{-2}	68·5	38·8
$SrCl_2$	10^{-3}	93·5	3·0
	10^{-2}	101·2	39·0

* Donor strain used in crosses was *Micrococcus lysodeikticus* ISU (*ade*$^+$-*1*).

Transformation of *S. flava* and *M. flavocyaneus* has shown that recipient competence is not specifically limited to *M. lysodeikticus* strains; however, this phenomenon appears not to occur in all micrococci related to *M. lysodeikticus*. Transformation may serve as a significant tool for future genetic studies of these organisms.

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