

## Screening of Amine Dehydrogenase of Microorganisms

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Screening of amine dehydrogenase from bacteria, yeasts and molds was done. In this paper, we report on histamine dehydrogenase and benzylamine dehydrogenase. Screening of amine dehydrogenase was carried out following the replica method. Twenty-nine microorganisms were grown on histamine plates under aerobic conditions. Ten microorganisms were grown on histamine plates under anaerobic conditions. On the other hand, sixteen microorganisms and two microorganisms were grown on benzylamine plates under aerobic and anaerobic conditions, respectively. No molds were grown on benzylamine plates.

### Introduction

Copper containing amino oxidase (EC 1.4.3.6) catalyzes the oxidation of various biogenic primary amines to corresponding aldehydes, ammonia and hydrogen peroxide<sup>1-3)</sup>. Recently, Tanizawa *et al.* have demonstrated, using the copper/topa quinone-less, inactive form of the recombinant phenethylamine oxidase from *Arthrobacter globiformis*, that the topa quinone cofactor is generated by copper-dependent autoxidation of the precursor Tyr<sup>4)</sup>. Therefore, amino dehydrogenase may have the same or another cofactor.

Thus, we did a screening of amine dehydrogenase from microorganisms.

### Materials and Methods

#### Microorganisms

Sixty-eight bacteria, 34 yeasts and 11 molds were used in this study.

#### Medium and cultivation condition

Cultivations of master plates were carried out as follows: Bacteria were cultured on nutrient agar plates containing 3.5% nutrient agar medium and 0.5% agar, with a pH of 7.0 at 28°C, lactic acid bacteria were cultured on BL agar plates containing 6% BL agar medium and 0.5% agar, with a pH of 7.2 at 37°C and yeasts

and molds were cultured on malt agar plates containing 2% malt extract and 2% agar with a pH of 5.5 at 28°C.

The histamine medium consisted of 0.1% histamine, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.1% MgSO<sub>4</sub> and 2% agar. The benzylamine medium consisted of 0.1% benzylamine, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.1% MgSO<sub>4</sub> and 2% agar. The medium of bacteria was adjusted to a pH of 7.0, and the medium of yeasts and molds was adjusted to a pH of 5.6.

#### Replica method

When colonies of microorganisms on the master plate were sufficiently grown, filter paper was put on the master plate, and the colonies were transferred to the filter paper. The filter paper was put on histamine plates and benzylamine plates to transfer colonies. Microorganisms on replica plates were cultured at 28°C for 3 days under both aerobic and anaerobic conditions. After cultivation, the growth of colonies on the histamine plates and benzylamine plates were checked.

### Results and Discussion

The effects of the growth of bacteria, yeasts and molds on the histamine and benzylamine medium are summarized in Table 1, 2 and 3, respectively. Figure 1 shows the colonies of yeasts on the histamine medium.

**Table 1.** Amine dehydrogenase activity of bacteria

Strain No.	Bacteria	Amine DH activity*			
		Histamine		Benzylamine	
		Aerobic	Anaerobic	Aerobic	Anaerobic
1	<i>Agrobacterium radiobacter</i> IAM 1526	—	—	—	—
2	<i>Agrobacterium tumefaciens</i> IAM B-26-1	—	—	+	—
3	<i>Alcaligenes faecalis</i> IAM B-141-1	—	—	+	—
4	<i>Alcaligenes polymorpha</i>	—	—	—	—
5	<i>Arthrobacter globiformus</i> IFO 12137	—	—	—	—
6	<i>Arthrobacter oxydans</i> IFO 12138	—	—	—	—
7	<i>Arthrobacter pascens</i> IFO 12139	—	—	—	—
8	<i>Arthrobacter simplex</i> IFO 3530	—	—	—	—
9	<i>Arthrobacter ureafaciens</i> IFO 12140	—	—	—	—
10	<i>Bacillus brevis</i> IFO 3331	+	—	+	—
11	<i>Bacillus cereus</i> IFO 3001	—	—	—	—
12	<i>Bacillus megaterrium</i> NI 8100 NTH B12	—	—	—	—
13	<i>Bacillus pumilus</i> IFO 3030	—	—	—	—
14	<i>Bacillus sphaericus</i> IFO 3525	—	—	—	—
15	<i>Bacillus brevis</i> IFO 3007	+	—	—	—
16	<i>Bacterium cadaveris</i> IFO3731	—	—	+	—
17	<i>Bacterium ketoglutamicus</i> SO(1)Shionogi Co., Ltd	+	—	+	—
18	<i>Bacterium orleanense</i> IFO 3259	+	—	+	—
19	<i>Brevibacterium</i> sp. P145 Phage host	—	—	—	—
20	<i>Brevibacterium</i> sp. P145 N. Kato	—	—	—	—
21	<i>Corynebacterium fascians</i> IAM 1079	—	—	—	—
22	<i>Corynebacterium glutamicum</i> No. 534 ATCC 13032	—	—	—	—
23	<i>Corynebacterium pseudodiphtheritium</i>	—	—	—	—
24	<i>Enterobacter aerogenes</i> IFO 3320	—	—	+	—
25	<i>Enterobacter cloacae</i> IAM 1221	—	—	+	—
26	<i>Erwinia arvideae</i> IFO 3830	—	—	—	—
27	<i>Erwinia carotovora</i> IFO 3380	+	—	+	—
28	<i>Escherichia coli</i> K12 IFO 3208	—	—	—	—
29	<i>Escherichia freundii</i> S-96	—	—	—	—
30	<i>Escherichia intermedia</i> A-21	—	—	—	—
31	<i>Klebsiella pneumoniae</i> IFO 3317	—	—	+	—
32	<i>Klebsiella pneumoniae</i> IFO 12009	—	—	—	—
33	<i>Klebsiella pneumoniae</i> IFO 12019	—	—	—	—
34	<i>Klebsiella pneumoniae</i> IFO 12932	—	—	—	—
35	<i>Klebsiella pneumoniae</i> IFO 13541	—	—	—	—
36	<i>Klebsiella pneumoniae</i> IFO 13703	—	—	—	—
37	<i>Micrococcus luteus</i> IFO 3763	—	—	—	—
38	<i>Micrococcus lysodeikticus</i> Fleming IFO 3333	—	—	—	—
39	<i>Micrococcus roseus</i> IFO 3764	—	—	—	—
40	<i>Micrococcus rubens</i> IFO 3768	—	—	—	—
41	<i>Proteus mirabilis</i> IFO 3849	—	—	—	—
42	<i>Proteus morgani</i> IFO 3168	—	—	—	—
43	<i>Proteus vulgaris</i> IFO 3988	+	—	—	—
44	<i>Pseudomonas fluorescens</i> IFO 3081	+	—	+	—
45	<i>Pseudomonas fragi</i> IFO 3458	—	—	—	—
46	<i>Pseudomonas graveolens</i> IFO 3460	+	—	+	—
47	<i>Pseudomonas iodium</i> IFO 3558	+	—	+	—
48	<i>Pseudomonas solanacearum</i> IFO 3509	+	—	+	—
49	<i>Pseudomonas striafaciens</i> IFO 3309	+	—	+	—
50	<i>Sarcina aurantiaca</i> IFO 3064	+	—	—	—
51	<i>Sarcina lutea</i> IFO 1099	—	—	—	—
52	<i>Serratia liquefaciens</i> IFO 12979	+	—	—	—
53	<i>Serratia marcescens</i> IFO 3054	+	—	—	—
54	<i>Serratia plymuthica</i> IFO 3055	+	—	+	+
55	<i>Staphylococcus aureus</i> IFO 3060	—	—	—	—
56	<i>Staphylococcus epidermidis</i> IFO 3762	—	—	—	—
57	<i>Bifidobacterium adolescentis</i> M101-4	—	—	—	—
58	<i>Bifidobacterium bifidum</i> A234-4	—	—	—	—
59	<i>Bifidobacterium breve</i> I-53-8	—	—	—	—
60	<i>Bifidobacterium infantis</i> I-10-5	—	—	—	—
61	<i>Bifidobacterium longum</i> M101-2	—	—	—	—
62	<i>Lactobacillus bulgaricus</i> IFO 13953	—	—	—	—
63	<i>Lactobacillus clerbueckii</i> IFO 3202	—	—	—	—
64	<i>Lactobacillus paracasei</i> IFO 3953	—	—	—	—
65	<i>Lactococcus cremoris</i> IFO 3427	—	—	—	—
66	<i>Lactococcus lactis</i> IFO 12007	—	—	—	—
67	<i>Pediococcus acidilactici</i> IFO 3888	—	—	—	—
68	<i>Streptococcus thermophilus</i> IFO 13957	—	—	—	+

\*, The abbreviation + in the table is reactive, — is not reactive.

Screening of Amine Dehydrogenase of Microorganisms

**Table 2.** Amine dehydrogenase activity of yeasts

Strain No.	Yeasts	Amine DH activity*			
		Histamine		Benzylamine	
		Aerobic	Anaerobic	Aerobic	Anaerobic
69	Awamori yeast (Sakamoto)	+	+	+	-
70	<i>Candida guilliermondii</i> IFO 0566	-	-	-	-
71	<i>Candida pelliculosa</i> IFO 0707	+	+	+	-
72	<i>Candida utilis</i> IFO 0619	-	-	-	-
73	<i>Cryptococcus albidus</i> IFO 0378	-	-	-	-
74	<i>Cryptococcus laurentii</i> IFO 0609	-	-	-	-
75	<i>Cryptococcus neoformans</i> IFO 0410	-	-	-	-
76	<i>Debaryomyces japonicus</i> IFO 0039	-	-	-	-
77	<i>Debaryomyces vini</i> Y.U.	+	-	-	-
78	<i>Endomyces capsularis</i> IFO 0672	-	-	-	-
79	<i>Endomyces decipiens</i> IFO 0102	+	+	-	-
80	<i>Hansenula miso</i> IFO 0146	+	+	-	-
81	<i>Hansenula suaveolens</i> IFO 0992	-	-	-	-
82	<i>Hansenula wingei</i> IFO 0976	-	-	-	-
83	<i>Kloeckera apiculata</i> IFO 0865	-	-	-	-
84	<i>Nadsonia fulvescens</i> IFO 0666	+	-	-	-
85	<i>Pichia anomala</i> IFO 0568	+	+	-	-
86	<i>Pichia orientalis</i> IFO 1279	+	-	-	-
87	<i>Pichia polymorpha</i> IFO 0195	+	-	-	-
88	<i>Pichia rhodanensis</i> IFO 1272	+	-	-	-
89	<i>Rhodotorula minuta</i> IFO 0387	-	-	-	-
90	<i>Rhodotorula rubra</i> IFO 0709	-	+	-	-
91	<i>Saccharomyces carlsbergensis</i> IFO 0641	+	+	-	-
92	<i>Saccharomyces rouxii</i> IFO 0487	-	+	-	-
93	<i>Saccharomyces sake</i> Kyoukai No.6	+	+	-	-
94	<i>Saccharomyces ludwigii</i> IFO 1043	-	-	-	-
95	<i>Saccharomycopsis fibuligere</i> IFO1744	-	-	-	-
96	<i>Torula rubra</i> var. <i>alpha</i>	-	-	-	-
97	<i>Torulopsis delbueckii</i> IFO 0428	-	-	-	-
98	<i>Torulopsis aeria</i>	-	+	-	-
99	<i>Torulopsis candida</i> IFO 0768	-	-	-	-
100	<i>Trichosporon cutaneum</i> IFO 1198	-	-	-	-
101	Wine yeast	-	-	-	-
102	<i>Zygosaccharomyces rouxii</i> IFO 0505	-	-	-	-

\*, The abbreviation + in the table is reactive, - is not reactive.

**Table 3.** Amine dehydrogenase activity of molds

Strain No.	Molds	Amine DH activity*			
		Histamine		Benzylamine	
		Aerobic	Anaerobic	Aerobic	Anaerobic
103	<i>Aspergillus niger</i> IFO 4414	-	-	-	-
104	<i>Aspergillus oryzae</i> IFO 4176	-	-	-	-
105	<i>Aspergillus parasiticus</i> IFO 5241	-	-	-	-
106	<i>Monascus purpureus</i> IFO 4478	+	-	-	-
107	<i>Mucor circinelloides</i> f. <i>circinelloides</i> IFO 4554	+	-	-	-
108	<i>Neurospora sitophila</i> IFO 4596	-	-	-	-
109	<i>Penicillium camembertii</i> IFO 5855	-	-	-	-
110	<i>Penicillium crysogenum</i>	-	-	-	-
111	<i>Rhizomucor pusillus</i> IFO 4578	-	-	-	-
112	<i>Rhizopus javanicus</i> IFO 5441	-	-	-	-
113	<i>Rhizopus oryzae</i> IFO 4706	-	-	-	-

\*, The abbreviation + in the table is reactive, - is not reactive.



**Fig. 1.** Growth of microorganisms on a plate containing histamine under aerobic conditions.

Twenty-nine microorganisms had grown on the histamine medium under aerobic conditions, and ten microorganisms had grown under anaerobic conditions.

In other words, approximately 25% of microorganisms cultured under aerobic conditions and approximately 10% of those cultured under anaerobic conditions had histamine dehydrogenase. Bacteria and molds had not formed colonies on the histamine medium under anaerobic conditions.

On the other hand, sixteen microorganisms had grown under aerobic conditions, and two microorganisms had grown on the benzylamine medium under anaerobic conditions. Yeasts did not form a colony on the benzylamine plate, so yeasts did not have benzylamine dehydrogenase under anaerobic conditions. In addition, no molds had grown on the same plate under both conditions.

### References

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