活動報告

平成１５年度中期構造生物学坂部プロジェクトの活動

運営委員会委員長
坂部知平

Ⅰ．ＳＢＳＰ用第一実験ステーションＢＬ６Ｂ

１．利用状況

平成１５年度前期のビームタイムは平成１５年１０月１日（水）午前９時に開始され１２月２４日（水）午前９時に終了した。BL6B及びBL6Cの予約状況をそれぞれ表１及び表２に示す。前回と同様bonus日(入射器のマシンスタディ)を予備日とし、それ以外の予備日は取らなかった。bonus timeは１週間前迄に急を要する要求がなければキャンセルを行った。

BL6BはR-AXIS Ⅳ++の導入により、検出器が複数になったため中期と同様後期開始時点では予約表に検出器の種類を示す記号を入れた。即ち、R-AXIS Ⅳ++を（R）、従来のLarge IPを（P）とした。しかし、終盤になって（P）に予約している人に問い合わせたところ、全員（R）で良いことが判明したので、表１に示されているように、最終的には全て（R）とした。

２．ビームラインアシスタント

今期のビームラインアシスタントの希望者は無かった。

３．装置の状況

１）ＢＬ６Ｂ

ビームラインの故障はなかった。測定装置はIP vaccume errorが２回出た。低温吹付け装置は全く正常に稼動した。

Station check sheetから状況を拾うと

１０月　９日	raxis1のpasswordがexpireされています。
１０月１７日	IP vaccume error (三浦さん対処済み)
１０月２２日	IP vaccume error (三浦さん対処済み)
１２月１８日	beam dump (20:20)

また使用波長を調べると

１０月２４日～２７日	0.9883Å、0.9815Å, 1.000 Å, 0.9712 Å MADdata収集
１１月　９日	0.979 Å
１１月２１日	1.04 Å

上記以外は全て1.0 Åの波長を使用している。

２）ＢＬ６Ｃ

ビームライン及び低温吹付け装置は共に正常に稼動した。

単色Ｘ線によるtime-resolved protein crystallographyのテストを行うため、ゴニオメータへ

ッドに付けるフローセル用金具を作成した。ポンプなどは未だ購入していない。

表１．平成15年度前期BL6Bビームタイム使用状況

10/ 1	WED	R	Kyowa_Hakko_Kogyo_c	(day)	- (night)
10/ 2	THU	R	Kyowa_Hakko_Kogyo_c	(day)	- (night)
10/ 3	FRI	R	Sankyo_Co._Ltd_c	(day)	- (night)
10/ 4	SAT	R	-	(day)	- (night)
10/ 5	SUN	R	-	(day)	- (night)
10/ 6	MON	R	machine_study	(day)	machine_study (night)
10/ 7	TUE	R	bonus_time	(day)	bonus_time (night)
10/ 8	WED	R	-	(day)	- (night)
10/ 9	THU	R	Yamanouchi_Pharm._c	(day)	- (night)
10/10	FRI	R	Daiichi_Pharm._c	(day)	Daiichi_Pharm._c (night)
10/11	SAT	R	-	(day)	- (night)
10/12	SUN	R	-	(day)	- (night)
10/13	MON	R	machine_study	(day)	machine_study (night)
10/14	TUE	R	-	(day)	- (night)
10/15	WED	R	Fujisawa_Pharm._c	(day)	- (night)
10/16	THU	R	Daiichi_Pharm._c	(day)	- (night)
10/17	FRI	R	Fujisawa_Pharm._c	(day)	- (night)
10/18	SAT	R	Eisai_Co._Ltd_c	(day)	Eisai_Co._Ltd_c (night)
10/19	SUN	R	Mizuno_Hiroshi_d	(day)	- (night)
10/20	MON	R	machine_study	(day)	machine_study (night)
10/21	TUE	R	-	(day)	- (night)
10/22	WED	R	Sankyo_Co._Ltd_c	(day)	- (night)
10/23	THU	R	Eisai_Co._Ltd_c	(day)	Eisai_Co._Ltd_c (night)
10/24	FRI	R	Sakabe_Kiwako_d	(day)	Sakabe_Kiwako_d (night)
10/25	SAT	R	Sasaki_Kyoyu_d	(day)	Sasaki_Kyoyu_d (night)
10/26	SUN	R	co_users	(day)	co_users (night)
10/27	MON	R	machine_study	(day)	machine_study (night)
10/28	TUE	R	bonus_time	(day)	bonus_time (night)
10/29	WED	R	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
10/30	THU	R	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
10/31	FRI	R	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
11/ 1	SAT	R	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
11/ 2	SUN	R	3GeV_single-bunch	(day)	3GeV_single-bunch (night)
11/ 3	MON	R	machine_study	(day)	machine_study (night)
11/ 4	TUE	R	bonus_time	(day)	bonus_time (night)
11/ 5	WED	R	Ajinomoto_Co._Inc_c	(day)	- (night)
11/ 6	THU	R	Yamanouchi_Pharm._c	(day)	- (night)
11/ 7	FRI	R	BERI_c	(day)	BERI_c (night)
11/ 8	SAT	R	BERI_c	(day)	- (night)
11/ 9	SUN	R	-	(day)	- (night)
11/10	MON	R	machine_study	(day)	machine_study (night)
11/11	TUE	R	Daiichi_Pharm._c	(day)	- (night)

11/12	WED	R	Banyu_Pharm._c	(day)	- (night)
11/13	THU	R	Sankyo_Co._Ltd_c	(day)	- (night)
11/14	FRI	R	Sankyo_Co._Ltd_c	(day)	- (night)
11/15	SAT	R	Mizuno_Hiroshi_d	(day)	- (night)
11/16	SUN	R	-	(day)	- (night)
11/17	MON	R	machine_study	(day)	machine_study (night)
11/18	TUE	R	-	(day)	- (night)
11/19	WED	R	Banyu_Pharm._c	(day)	- (night)
11/20	THU	R	Ajinomoto_Co._Inc_c	(day)	- (night)
11/21	FRI	R	co_users	(day)	co_users (night)
11/22	SAT	R	co_users	(day)	co_users (night)
11/23	SUN	R	-	(day)	- (night)
11/24	MON	R	machine_study	(day)	machine_study (night)
11/25	TUE	R	bonus_time	(day)	bonus_time (night)
11/26	WED	R	-	(day)	- (night)
11/27	THU	R	-	(day)	- (night)
11/28	FRI	R	Liang_Dong-cai_b	(day)	Liang_Dong-cai_b (night)
11/29	SAT	R	Chang_Wen-rui_b	(day)	Chang_Wen-rui_b (night)
11/30	SUN	R	-	(day)	- (night)
12/ 1	MON	R	machine_study	(day)	machine_study (night)
12/ 2	TUE	R	bonus_time	(day)	bonus_time (night)
12/ 3	WED	R	-	(day)	- (night)
12/ 4	THU	R	Fujisawa_Pharm._c	(day)	- (night)
12/ 5	FRI	R	-	(day)	- (night)
12/ 6	SAT	R	-	(day)	- (night)
12/ 7	SUN	R	overseas_users_a	(day)	overseas_users_a (night)
12/ 8	MON	R	machine_study	(day)	machine_study (night)
12/ 9	TUE	R	-	(day)	- (night)
12/10	WED	R	-	(day)	- (night)
12/11	THU	R	Ajinomoto_Co._Inc_c	(day)	- (night)
12/12	FRI	R	co_users	(day)	- (night)
12/13	SAT	R	-	(day)	Kitadokoro_Kengo_d (night)
12/14	SUN	R	Kitadokoro_Kengo_d	(day)	- (night)
12/15	MON	R	machine_study	(day)	machine_study (night)
12/16	TUE	R	Yamanouchi_Pharm._c	(day)	- (night)
12/17	WED	R	Yamanouchi_Pharm._c	(day)	- (night)
12/18	THU	R	Sankyo_Co._Ltd_c	(day)	Daiichi_Pharm._c (night)
12/19	FRI	R	Tada_Toshiharu_d	(day)	Tada_Toshiharu_d (night)
12/20	SAT	R	Fujii_Ikuo_d	(day)	Fujii_Ikuo_d (night)
12/21	SUN	R	Ihara_Hide_d	(day)	Ihara_Hide_d (night)
12/22	MON	R	co_users	(day)	co_users (night)
12/23	TUE	R	-	(day)	- (night)

4．ＢＬ６Ｃの成果

ＢＬ６Ｃを使用して世界記録が出たので紹介する。

坂部知平、名大・環境学研究科・佐々木教祐教授等によりヒトリコンビナント２亜鉛イン

表２．平成１５年度前期 BL6C ビームタイム使用状況

10/ 1 WED A	Camera_maintenance (day)	- (night)
10/ 2 THU A	Camera_maintenance (day)	- (night)
10/ 3 FRI A	Camera_maintenance (day)	- (night)
10/ 4 SAT A	Camera_maintenance (day)	- (night)
10/ 5 SUN A	Camera_maintenance (day)	- (night)
10/ 6 MON A	machine_study (day)	machine_study (night)
10/ 7 TUE A	bonus_time (day)	bonus_time (night)
10/ 8 WED A	Sasaki_Kyoyu_d (day)	- (night)
10/ 9 THU A	Sasaki_Kyoyu_d (day)	- (night)
10/10 FRI A	co_users (day)	- (night)
10/11 SAT A	co_users (day)	- (night)
10/12 SUN A	- (day)	- (night)
10/13 MON A	machine_study (day)	machine_study (night)
10/14 TUE A	bonus_time (day)	bonus_time (night)
10/15 WED A	- (day)	- (night)
10/16 THU A	- (day)	- (night)
10/17 FRI A	Eisai_Co._Ltd_c (day)	- (night)
10/18 SAT A	- (day)	- (night)
10/19 SUN A	- (day)	- (night)
10/20 MON A	machine_study (day)	machine_study (night)
10/21 TUE A	bonus_time (day)	bonus_time (night)
10/22 WED A	- (day)	- (night)
10/23 THU A	- (day)	- (night)
10/24 FRI A	Eisai_Co._Ltd_c (day)	- (night)
10/25 SAT A	Eisai_Co._Ltd_c (day)	- (night)
10/26 SUN A	Eisai_Co._Ltd_c (day)	- (night)
10/27 MON A	machine_study (day)	machine_study (night)
10/28 TUE A	bonus_time (day)	bonus_time (night)
10/29 WED A	3GeV_single-bunch (day)	3GeV_single-bunch (night)
10/30 THU A	3GeV_single-bunch (day)	3GeV_single-bunch (night)
10/31 FRI A	3GeV_single-bunch (day)	3GeV_single-bunch (night)
11/ 1 SAT A	3GeV_single-bunch (day)	3GeV_single-bunch (night)
11/ 2 SUN A	3GeV_single-bunch (day)	3GeV_single-bunch (night)
11/ 3 MON A	machine_study (day)	machine_study (night)
11/ 4 TUE A	bonus_time (day)	bonus_time (night)
11/ 5 WED A	Sasaki_Kyoyu_d (day)	- (night)
11/ 6 THU A	Sakabe_Kiwako_d (day)	- (night)
11/ 7 FRI A	Sakabe_Kiwako_d (day)	- (night)
11/ 8 SAT A	Sakabe_Kiwako_d (day)	- (night)
11/ 9 SUN A	- (day)	- (night)
11/10 MON A	machine_study (day)	machine_study (night)
11/11 TUE A	bonus_time (day)	bonus_time (night)
11/12 WED A	- (day)	- (night)
11/13 THU A	- (day)	- (night)
11/14 FRI A	- (day)	- (night)

11/15 SAT A	Sasaki_Kyoyu_d (day)	- (night)
11/16 SUN A	Sasaki_Kyoyu_d (day)	- (night)
11/17 MON A	machine_study (day)	machine_study (night)
11/18 TUE A	bonus_time (day)	bonus_time (night)
11/19 WED A	- (day)	- (night)
11/20 THU A	- (day)	- (night)
11/21 FRI A	- (day)	- (night)
11/22 SAT A	- (day)	- (night)
11/23 SUN A	- (day)	- (night)
11/24 MON A	machine_study (day)	machine_study (night)
11/25 TUE A	bonus_time (day)	bonus_time (night)
11/26 WED A	- (day)	- (night)
11/27 THU A	- (day)	- (night)
11/28 FRI A	- (day)	- (night)
11/29 SAT A	- (day)	- (night)
11/30 SUN A	- (day)	- (night)
12/ 1 MON A	machine_study (day)	machine_study (night)
12/ 2 TUE A	bonus_time (day)	bonus_time (night)
12/ 3 WED A	Liang_Dong-cai_b (day)	Liang_Dong-cai_b (night)
12/ 4 THU A	Liang_Dong-cai_b (day)	Liang_Dong-cai_b (night)
12/ 5 FRI A	Liang_Dong-cai_b (day)	Liang_Dong-cai_b (night)
12/ 6 SAT A	- (day)	- (night)
12/ 7 SUN A	- (day)	- (night)
12/ 8 MON A	machine_study (day)	machine_study (night)
12/ 9 TUE A	bonus_time (day)	bonus_time (night)
12/10 WED A	Chang_Wen-rui_b (day)	Chang_Wen-rui_b (night)
12/11 THU A	Chang_Wen-rui_b (day)	Chang_Wen-rui_b (night)
12/12 FRI A	Chang_Wen-rui_b (day)	Chang_Wen-rui_b (night)
12/13 SAT A	- (day)	- (night)
12/14 SUN A	- (day)	- (night)
12/15 MON A	machine_study (day)	machine_study (night)
12/16 TUE A	bonus_time (day)	bonus_time (night)
12/17 WED A	- (day)	- (night)
12/18 THU A	Daiichi_Pharm._c (day)	- (night)
12/19 FRI A	- (day)	- (night)
12/20 SAT A	- (day)	- (night)
12/21 SUN A	- (day)	- (night)
12/22 MON A	- (day)	- (night)
12/23 TUE A	- (day)	- (night)

スリン結晶から１００Ｋで分解能0.8Åのデータ収集が行われ、リガンドによりredistributeされた亜鉛イオンのｄ電子が観測された。同氏等は1984年にもブタ２亜鉛インスリンの分解能1.2Åデータにより同様の電子密度を得ているが、当時はイオンの中心からｄ電子のピークまでの距離が1.4Å有ったのに対し、今回は1.0Åであった。予想距離は0.6Åである。BL6Cの装置はＸ線の波長を0.9Åにすれば、分解能0.6Åまでのデータを測定できる。尚一層良いデータを収集する予定である。尚、この結果は平成１５年１１月１９～２１日に原研で行われた国際

シンポジウム（The 5th International Symposium on 'Development of New Structural Biology Including Hydrogen and Hydration' in Organized Research Combination System）で発表された。

Ⅱ．コンピュータ関係

コンピュータネットワーク及びデータサーバが更新された。詳細は佐々木教祐氏が記載された本誌　6 頁を参照されたい。

Ⅲ．委員会報告

１．運営委員会

第１０回運営委員会が下記のように開催され平成１４年度決算及び平成１５年度予算が承認された。詳細は本誌１頁第１０回委運営委員会を参照されたい。

日　時　：　平成１６年１月１５日（木）、　１１：００～１３：００
場　所　：　高エネルギー加速器研究機構内SBSPハウス２階
参加者数：　１４名

２．．編集委員会

編集委員会はメールにより連絡を取り合い、下記の事項を決定し次号発行の作業を行った。
１）曽我部智委員が辞任したため空席となっていた委員に松本拓男氏（三共）が就任された。
２）構造生物Vol.19,No.1の最終チェック並びに印刷等のスケジュール確認が行われた。

３．行事委員会

ＳＢＳＰ参加企業成果報告会が下記の様に開催された
日　時　：　平成１６年１月１５日（木）１３：３０～１６：３０
場　所　：　高エネルギー加速器研究機構４号館１階セミナーホール
報告企業：　味の素㈱、協和発酵工業㈱、キリンビール㈱、三共㈱、生物分子工学研究所、第一製薬㈱、中外製薬㈱、万有製薬㈱、藤沢薬品工業㈱、三菱化学㈱
参加者数：　３１名

各企業としても発表可能なぎりぎりの線まで発表が行われ、発表のレベルは高かった。質疑討論も活発で、極めて有意義な成果報告会であった。

Ⅳ．業績紹介

1.　祥雲　弘文（筑波大学、現在東京大学）

Oxygen requirement for denitrification by the fungus *Fusarium oxysporum*
Arch Microbiol. **175**, 19-25, (2001)

Zhemin Zhou, Naoki Takaya, Maria Antonina C. Sakairi, Hirofumi Shoun,
Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Summary

The effects of dioxygen (O_2) on the denitrification activity of the fungus *Fusarium*

oxyporum MT-811 in fed-batch culture in a stirred jar fermentor were examined. The results revealed that fungal denitrifying activity requires a minimal amount of O_2 for induction, which is repressed by excess O_2. The optimal O_2 supply differed between the denitrification substrates : 690 μmol O_2 h^{-1} (g dry cell wt.)$^{-1}$ for nitrate (NO_3^-) and about 250 μmol O_2 h^{-1} (g dry cell wt.)$^{-1}$ for nitrite (NO_2^-). The reduction of NO_3^- required more O_2 than that of NO_2^-. With an optimal O_2 supply, 80% and 52% of nitrogen atoms in NO_3^- and NO_2^-, respectively, were recovered as the denitrification product N_2O. These features of *F. oxysporum* differ from those of bacterial denitrifiers that work exclusively under anoxic conditions. The denitrification activity of *F. oxysporum* MT-811 mutants with impaired NO_3^- assimilation was about double that of the wild-type strain, suggesting competition for the substrate between assimilatory and dissimilatory types of NO_3^- reduction. These results showed that denitrification by *F. oxysporum* has unique features, namely, a minimal O_2 requirement and competition with assimilatory NO_3^-.

２． 祥雲 弘文（筑波大学、現在東京大学）

A Novel C1-Using Denitrifier *Alcaligenes* sp. STC1 and Its Genes for Copper-containing Nitrite Reductase and Azurin

Biosci. Biotechnol. Biochem., **65**(5), 1206-1210, (2001)

Shigeru Ozeki, Ikuko Baba, Naoki Takaya, and Hirofumi Shoun
Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Summary
A novel denitrifier *Alcaligenes* sp. STC1 was identified. The strain efficiently denitrifies under an atmosphere of 10% oxygen (O_2) where *Paracoccus denitrificans*, one of the most studied aerobic denitrifiers, had less denitrifying activity, indicating that the strain has an O_2-torelant denitrifying system. It denitrified by using C1-carbon sources such as formate and methanol as well as glucose, glycerol, and succinate. The genes for the copper-containing nitrite reductase and azurin of this C1-using denitrifier were cloned. Their predicted products of them were similar to those of their counterparts and the maximum similarities were 90% and 92%, respectively.

３．、祥雲 弘文（東京大学）

Nitrate Reductase-Formate Dehydrogenase Couple Involved in the Fungal Denitrification by *Fusarium oxysporum*

J. Biochem. **131**, 579-586, (2002)

Hiromasa Uchimura[1], Hitoshi Enjoji[1], Takafumi Seki[1], Ayako Taguchi[1], Naoki Takaya[1], and Hirofumi Shoun[2]
[1]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572; Japan and [2]Department of Biotechnology, Graduate School of Agricultural and Life Sciences The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

Summary
Dissimilatory nitrate reductase (Nar) was solubilized and partially purified from the large particle (mitochondrial) fraction of the denitrifying fungus *Fusarium oxysporum* and characterized. Many lines of evidence showed that the membrane-bound Nar is distinct from the soluble, assimilatory nitrate reductase. Further, the spectral and other properties of the fungal Nar were similar to those of dissimilatory Nars of *Escherichia coli* and denitrifying bacteria, which are comprised of a molybdoprotein, a cytochrome *b*, and an iron-sulfur protein. Formate-nitrate oxidoreductase activity was also detected in the mitochondrial fraction, which was shown to arise from the coupling of formate dehydrogenase (Fdh), Nar, and a ubiquinone/ubiquinol pool. This is the first report of the occurrence in a eukaryote of Fdh that is associated with the respiratory chain. The coupling with Fdh showed that the fungal Nar system is more similar to that involved in the nitrate respiration by *Escherichia coli* than that in the bacterial denitrifying system. Analyses of the mutant species of *F oxysporum* that were defective in Nar and/or assimilatory nitrate reductase conclusively showed that Nar is essential for the fungal denitrification.

4． 祥雲 弘文（東京大学）

Codenitrification and Denitrification are Dual Metabolic Pathways through Which Dinitrogen Evolves from Nitrate in *Streptomyces antibioticus*

Journal of Bacteriology, **148,** 2963-2968, (June 2002)

Yasuyuki Kumon[1], Yasuyuki Sasaki[1], Isao Kato[1], Naoki Takaya[1], Hirofumi Shoun[2] and Teruhiko Beppu[3]
[1]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, [2]Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, and [3]Department of Applied Biological Sciences, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252-8510, Japan

Summary
We screened actinomycete strains for dinitrogen (N_2)-producing activity and discovered that *Streptomyces antibioticus* B-546 evolves N_2 and some nitrous oxide (N_2O) from nitrate (NO_3^-). Most of the N_2 that evolved from the heavy isotope ($[^{15}N]NO_3^-$) was $^{15}N^{14}N$, indicating that this nitrogen species consists of two atoms, one arising from NO_3^- and the other from different sources. This phenomenon is similar to codenitrification in fungi. The strain also evolved less, but significant, amounts of $^{15}N^{15}N$ from $[^{15}N]NO_3^-$ in addition to $^{15}N^{15}NO$ with concomitant cell growth. Prior to the production of N_2 and N_2O, NO_3^- was rapidly reduced to nitrite (NO_2^-) accompanied by distinct cell growth, showing that the actinomycete strain is a facultative anaerobe that depends on denitrification and nitrate respiration for anoxic growth. The cell-free activities of denitrifying enzymes could be reconstituted, supporting the notion that the $^{15}N^{15}N$ and $^{15}N^{15}NO$ species are produced by

denitrification from NO_3^- via NO_2^-. We therefore demonstrated a unique system in an actinomycete that produces gaseous nitrogen (N_2 and N_2O) through both denitrification and codenitrification. The predominance of codenitrification over denitrification along with oxygen tolerance is the key feature of nitrate metabolism in this actinomycete.

5．祥雲　弘文（東京大学）

Ammonia Fermentation, a Novel Anoxic Metabolism of Nitrate by Fungi

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Zhemin Zhou[1], Naoki Takaya[1], Akira Nakamura[1], Masashi Yamaguchi[2], Kanji Takeo[2], and Hirofumi Shoun[3]
[1]Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, the [2]Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-8673, and the [3]Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

Summary
The induction of fungal denitrification by *Fusarium oxysporum* requires a minimal amount of O_2, although excess O_2 completely represses this process (Zhou, Z., Takaya, N., Sakairi, M. A. C., and Shoun, H. (2001) *Arch. Microbiol.* 175, 19-25). Here we describe another metabolic mechanism of nitrate in fungal cells, termed ammonia fermentation, that supports growth under conditions more anoxic than those of denitrification. The novel nitrate metabolism of eukaryotes consists of the reduction of nitrate to ammonium coupled with the catabolic oxidation of electron donors to acetate and substrate-level phosphorylation. *F. oxysporum* thus has two pathways of dissimilatory nitrate reduction that are alternatively expressed in response to environmental O_2 tension. *F. oxysporum* prefers O_2 respiration when the O_2 supply is sufficient. We discovered that this fungus is the first eukaryotic, facultative anaerobe known to express one of three distinct metabolic energy mechanisms closely depending on environmental O_2 tension. We also showed that ammonia fermentation occurs in many other fungi that are common in soil, suggesting that facultative anaerobes are widely distributed among fungi that have been considered aerobic organisms.